

American Journal of Clinical Pathology

S. E. Gould, M. D., *Editor*

Volume 17

Baltimore
The Williams & Wilkins Company
1947

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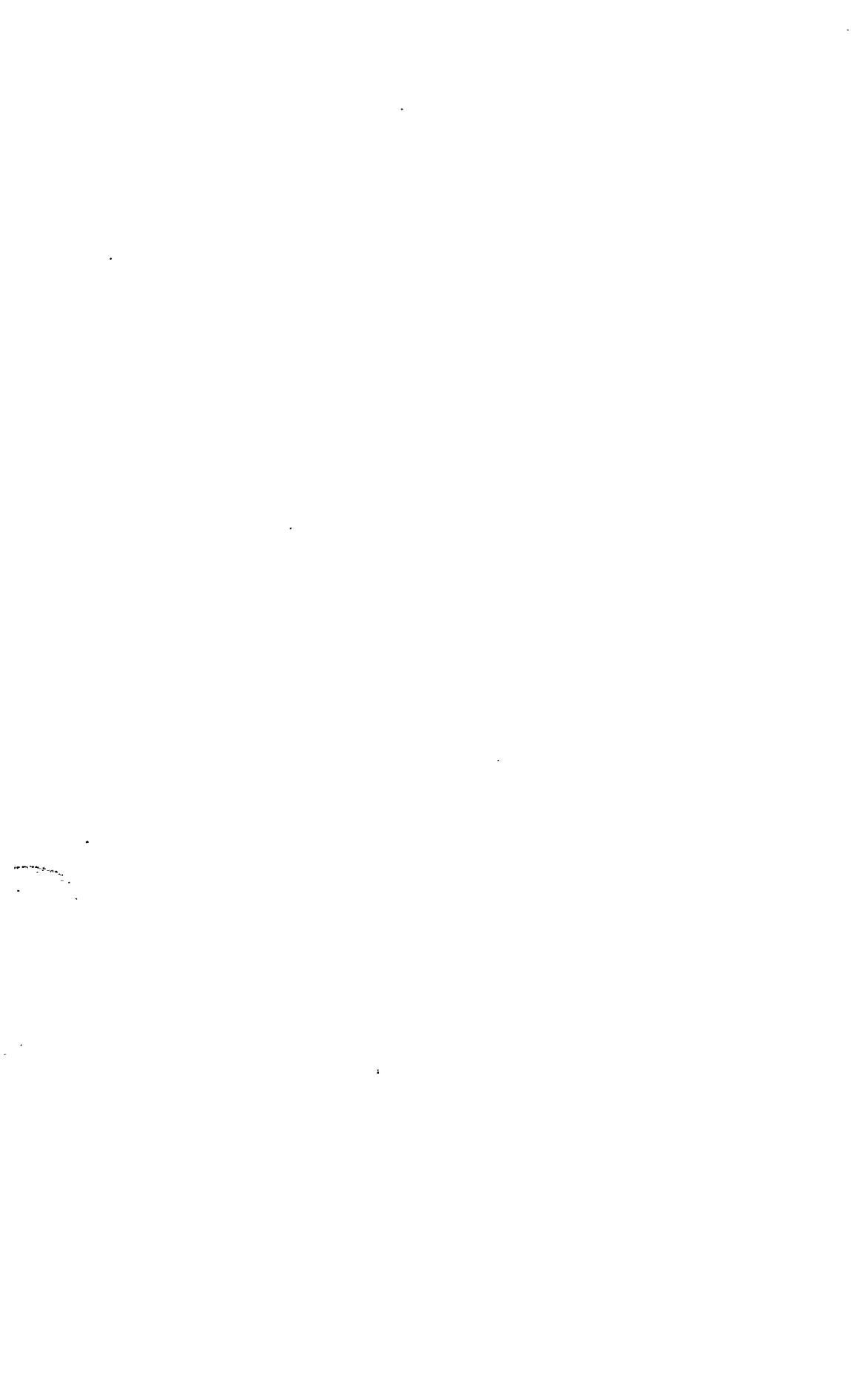
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SELECTION OF SALMONELLA AND SHIGELLA CULTURES FOR SEROLOGIC CLASSIFICATION*

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A large proportion of the microorganisms sent to various laboratories for confirmation as *Salmonella* or *Shigella* types belong to neither group, but are paracolon, coliform, or other enteric bacteria. For this reason, the writers have been urged¹² to present a revision of an article which appeared in a military journal, published in Italy,¹³ dealing with procedures which aid in the elimination of paracolon and *Proteus* cultures in the laboratory in which they are first isolated. The writers were then assigned to the 15th Medical General Laboratory, stationed in Italy. They found that of the cultures submitted to them for typing as salmonellae or shigellae, 39 per cent were paracolon, *Proteus*, or other bacteria.

During a twenty-one month period, some 24,000 fecal specimens and rectal swabs from patients and food handlers (civilian and Army personnel) were examined bacteriologically. The techniques which were employed in the examination of these specimens were outlined in the first publication and will be presented here in a somewhat revised form. The subject will first be treated in a general way and then the differentiation of *Salmonella* and *Shigella* cultures will be discussed separately.

GENERAL

There are several points to be considered in the original treatment of a specimen. In both bacillary dysentery and gastro-enteritis or enteric fever caused by the salmonellae, it is important to make cultures in the acute stage of the disease, since the number of microorganisms in the stool diminishes rapidly and late cultures are likely to be negative. Early in the infection the patient's stools are formed. This is followed by a series of watery evacuations which contain shreds of mucus, and often, especially in bacillary dysentery, macroscopic blood. If the mucous shreds are washed in physiologic saline and then cultured directly by streaking over the surface of the plating medium, the infecting micro-organism is usually recovered in almost pure culture. Also, it is important that the specimen be cultured just as soon as possible after collection. One of the best procedures is to deliver the stool in the pan directly to the laboratory, so that the bacteriologist can select suitable portions for microscopic examination.

* This paper is, in part, based on work done in the 15th Medical General Laboratory, United States Army. Received for publication, July 31, 1946.

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and cultural work. If this cannot be done, some other means for rapid delivery and prompt treatment of the specimen must be devised. When conditions are such that it is impossible to culture all specimens immediately, it is recommended that a suitable portion, containing mucous shreds, if present, be placed in a preserving medium until such time as it can be cultured conveniently. The preservative employed by Sachs (see Boyd⁵) can be recommended; other such mediums have been reviewed by Bangsang and Elliott.²

When it is possible to do so, it is often a distinct advantage to collect rectal swabs for cultural study. By this method, an experienced team of two or three workers can collect and culture several hundred specimens in a short period of time. The technic is also useful for the examination of food handlers and for collecting specimens from dispensary or office patients.

In chronic cases where the stool is negative, swabs taken directly from the lesions at proctoscopic examination often reveal the inciting microorganism in nearly pure culture.

Plating mediums. Several good plating mediums are available. The highly selective Shigella-Salmonella Agar (Difco) and Desoxycholate Citrate Agar (BBL) are excellent and have the advantage that large amounts of inoculum may be used. Brilliant green agar, as recommended by Kauffmann,¹⁸ is widely used in the isolation of salmonellae and bismuth sulfite agar surpasses all others in detecting typhoid bacilli.⁹ Fecal material or mucus on a swab may be streaked over the entire surface of the plate since coliform organisms are greatly inhibited. In using these mediums, however, great care must be exercised in picking colonies, because, although the coliform bacteria on the plate are inhibited, they are not necessarily dead. If colonies are not carefully picked, extraneous microorganisms may be transferred to the triple sugar medium. This point is stressed because Kligler's slants are frequently received which have two or three different microorganisms present, owing to carelessness in fishing colonies. In routine work on hospital cases, it is advisable to employ a differential plating medium such as eosin-methylene-blue or MacConkey agar in addition to selective mediums. This procedure affords a better idea of the general intestinal flora and allows isolation of an occasional pathogen which may not grow on the selective mediums.

Enrichment mediums. Selenite-F medium (BBL) is probably the medium of choice for general work. For salmonellae, investigators prefer tetrathionate bouillon. Galton and Quan¹⁶ reported an increase of 164 per cent in finding Salmonella owing to the efficacy of the combination of tetrathionate bouillon and brilliant green agar. Tetrathionate medium, however, is not considered a favorable medium for shigellae, and these microorganisms usually can be cultured from Selenite-F broth. The procedure recommended is to inoculate the primary plating medium with fecal material by means of cotton swabs made on half an applicator stick and then to place the swab containing from 0.1 to 1.0 gm. of feces in a tube of Selenite-F enrichment medium. The specimen must be emulsified in the enrichment broth. A large inoculum should be used with enrichment medium; the coliform bacteria are inhibited and the enteric pathogens have an opportunity to increase in number. The Selenite-F and tet-

rathionate mediums are incubated from sixteen to eighteen hours and if the primary plates are negative, several large loopfuls of the broth are cultured on an additional (secondary) S.S., brilliant green agar, or desoxycholate citrate-agar plate. Bismuth sulfite agar plates heavily seeded are frequently employed without any inoculation of enrichment broth to isolate *Salmonella typhi*.

In acute cases of both salmonellosis and bacillary dysentery (shigellosis), the primary plates are usually positive, so that the Selenite-F medium, or tetrathionate bouillon, is of greatest value with specimens collected after the acute phase in chronic cases and in carrier studies where the number of pathogens in the feces is likely to be small. Incidentally, it might be well to note that enrichment mediums may be used to advantage for cultures of blood, urine and gall-bladder drainage in cases in which a *Salmonella* type is considered as a possible etiologic agent.

Differential Mediums. When plates are obtained which contain colonies of nonlactose fermenting bacteria, several such colonies are transferred to Kligler's or Krumwiede's medium. We employ routinely a modified Kligler's medium made by adding 1.0 per cent sucrose to Kligler's Iron Agar (Difco); this medium also may be considered as a modification of Krumwiede's Triple Sugar medium. The modified Kligler's medium is similar in reaction to that recently described by Hajna.¹⁷ (The Triple Sugar Iron medium of Hajna is available from Baltimore Biological Laboratory and a similar one is available from Difco Laboratories.) After overnight incubation at 37 C., these slants are examined and those which have acid and gas throughout are recorded and discarded. Occasionally, slants are seen which show acid only throughout and these are usually anaerogenic coliforms. Sometimes, there are gram-positive cocci, and a fair number of these are received for confirmation as shigellae. Kligler's or Triple Sugar Iron (TSI) slants which exhibit the typical acid butt, with or without gas, and alkaline slants are inoculated on Christensen's⁷ urea medium for the elimination of *Proteus* cultures. The latter medium is incubated from two to four hours at 37 C., at the end of which time a preliminary reading is made. Those specimens that are negative at the preliminary reading are then tested with *Salmonella* and *Shigella* antisera by the spot plate technic (*vide infra*). Following the preliminary reading, the urea medium is reincubated; positive reactions are usually complete at twenty-four hours' incubation and may be recorded and discarded, if desired. Negative cultures are incubated at least forty-eight hours, since it has been found that certain bacteria of the paracolon *Aerobacter* group give a weak delayed reaction. This fact aids in the differentiation of these cultures. The salmonellae and shigellae are uniformly negative. The formula for Christensen's urea medium and details of its use are given by Ewing.¹⁴

Biochemical and physiologic reactions. The final step in the examination of cultures is the study of their biochemical and physiologic reactions. This is the only method at present available for the final elimination of paracolon bacteria. Cultures thought to be *Salmonella* or *Shigella* types should be confirmed as such by the application of a few important biochemical tests before they are submitted for typing.

For indol and hydrogen sulfide tests we employed 2 per cent Bacto peptone plus 0.5 per cent sodium chloride and 0.3 per cent beef extract in tap water. Single tubes were used and, after inoculation, a strip of oxalic acid impregnated paper and a strip of lead acetate paper were suspended inside the tube and held in place by a cotton stopper. Tests for indol may also be made with Kovac's reagent after the papers have been read, if so desired.

We recommend the use of a semisolid medium for determination of motility among the enteric microorganisms. The method is more accurate than microscopic examination, especially in the case of sluggishly motile bacteria. The motility medium described by Edwards and Bruner¹¹ is an excellent one; or, tryptone water, containing 0.25 per cent agar, may be employed.

The interpretation of the above physiologic reactions regarding *Salmonella* and *Shigella* cultures as well as the reactions in carbohydrate broths are discussed below under the separate headings *Salmonella* and *Shigella*.

SALMONELLA

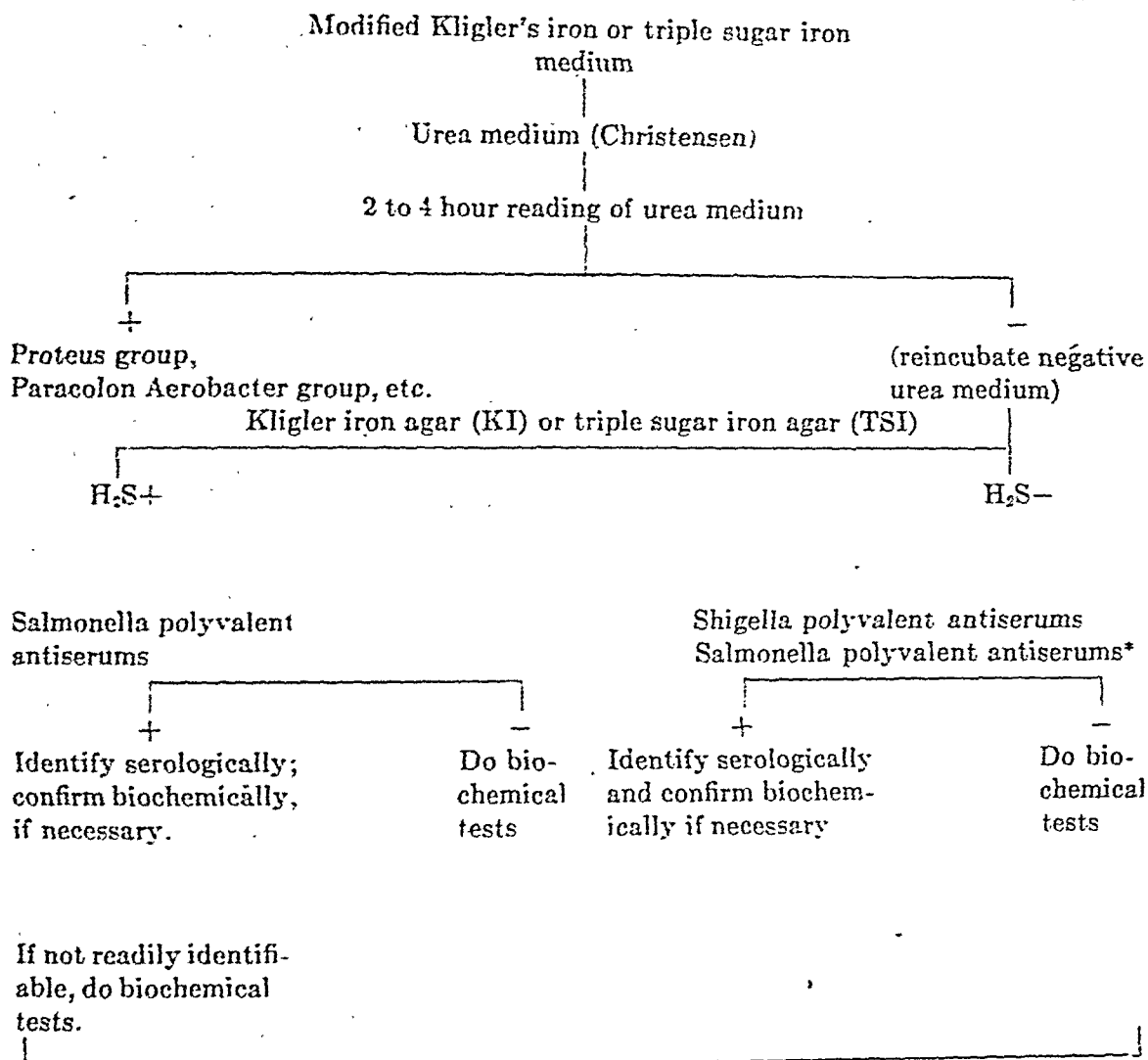
During twenty-one months of operation in Italy, the Veterinary Section of the 15th Medical General Laboratory received 1789 cultures for classification as *Salmonella*. Among these, 757 (42 per cent) were proved not to be *Salmonella* types. The purpose of this writing is to describe certain biochemical and serologic tests by which many of those non-*Salmonella* organisms can be readily screened out by the laboratory in which they are isolated. Although no absolute schema can be evolved for the immediate deletion of all nonmembers of the genus, it is possible to employ some routine procedures which will eliminate a large number of them. Since the group is rather broad and has been extended to include a large number of serologically related microorganisms, it is not desirable to adhere too closely to any set rules in choosing cultures for confirmation as *Salmonella*. In certain instances, the final identification is based on some characteristic of the microorganism, which is almost impossible to establish in the field laboratory.

In order to draw a line of demarcation between the genus *Salmonella* and related types, it is necessary to frame a definition. The one generally accepted for the genus is as follows:¹¹ *Salmonella* is "a large genus of serologically related, gram-negative and non sporing bacilli, 0.4 to 0.6 microns by 1 to 3 microns in usual dimensions, but occasionally forming short filaments; showing, with certain exceptions, a motile peritrichous phase in which they normally occur; in fact adhering to the pattern of *B. typhosus* in staining properties and morphology. Failing to ferment sucrose or to clot milk and rarely fermenting lactose, liquefying gelatin or producing indol, they regularly attack glucose with, but occasionally without, gas production. All the known species are pathogenic for man, animals, or both."

In selecting a culture to be submitted for confirmation as *Salmonella*, some practical tests of a biochemical nature and others of serologic character are employed. Since, in our comments above, we have carried the identification of enteric bacteria to the stage of modified-Kligler's medium and have tested the

TABLE 1

OUTLINE OF PROCEDURE FOR IDENTIFICATION OF SALMONELLA AND SHIGELLA CULTURES



	<i>Salmonella</i>	<i>Shigella</i>
Glucose	+	+
Lactose	-	V (delayed)†
Sucrose	-	V (delayed)
Salicin	-	-
Mannitol	not needed	V
Citrate	not needed	-
Motility	not needed	-
H ₂ S(paper)	+	V
Indol (paper)	-	V

* Occasional *Salmonella* cultures may fail to produce H₂S in Kligler's medium. Also certain shigellae and salmonellae cross-agglutinate, a fact which aids in their recognition (see text).

† Indicates a positive reaction, urea, hydrolysis, H₂S production, carbohydrate utilization, or agglutination in antiserum; -, no reaction; v, that results vary with different cultures; and V, that, if there is a reaction, it is usually delayed 48 hours or longer.

bacteria with urea agar, we will continue our screening at that point. Ordinarily, *Salmonella* suspects are hydrogen sulfide producers and do not form indol. They ferment glucose, but do not ferment lactose, salicin or sucrose. Accordingly, the ability of the selected culture to conform to these reactions should be investigated.

Tests for hydrogen sulfide and indol are made as indicated above in the general discussion. Such tubes should be incubated for forty-eight hours before being discarded. *Salmonella cholerae-suis* (American type) does not produce hydrogen sulfide by this procedure, nor does *Salmonella berta* or *Salmonella senftenberg* var. *newcastle*. We encountered none of those serologic types in the Mediterranean area. The other members of the genus, including *Salmonella paratyphi* A, blacken lead acetate paper. Certain cultures of *Salmonella eastbourne* produce indol (Kovac's reagent), but members of the genus are not known to produce a pink color on oxalic acid paper under the conditions of this test.

Fermentation tests are conducted in nutrient broth containing brom-cresol-purple or Andrade's indicator and 1 per cent of the fermentable substances, except for salicin, which is employed in 0.5 per cent concentration. *Salmonella* suspects should ferment glucose with the formation of acid and usually of gas. *Salmonella gallinarum*, *Salmonella typhi* (*Eberthella typhosa*) and, occasionally, cultures of *Salmonella typhi-murium* are anaerogenic. The presumptive *Salmonella* type should not ferment salicin, sucrose, or lactose. Lactose fermenting *Salmonella* have been described but they are extremely rare and in the examination of 1032 cultures by this laboratory none was encountered. An outline of a procedure for the identification of salmonellae and shigellae which includes the biochemical and physiologic reactions regarded as "minimum", is given in Table 1. Adonitol may be substituted for, or used in conjunction with salicin, since the salmonellae do not utilize this substance. (For additional physiologic reactions of the *Salmonella* group, see Kauffmann¹⁸ or Topley and Wilson.²²)

As a rule, fermentation tests should be incubated at 37 C. for two weeks before being discarded. If this plan is followed and only those cultures which exhibit the above listed characteristics are submitted, it will be observed that only a small percentage will fail in classification as *Salmonella* types. The elimination of this small percentage is based on the lack of any serologic relationship to the known *Salmonella* bacteria, combined with ability to ferment salicin, lactose, or sucrose on prolonged incubation. Whereas it may be necessary to wait several months before biochemical behavior warrants a negative report, a microorganism which bears the antigens of an established *Salmonella* type can be identified readily and quickly.

Where it is necessary to submit cultures for *Salmonella* confirmation after only a short period of observation, the same tests should be conducted to rule out those bacteria which quickly show their nonconformity to the above biochemical pattern. Sealing the fermentation tubes with corks dipped in paraffin often will increase the rapidity with which a sugar is attacked by a bacterium potentially able to do so.

Frequently, it is desirable to combine rapid serologic tests along with biochem-

ical methods in order to decide whether a particular culture warrants immediate confirmation as a type of *Salmonella*. A polyvalent anti-*Salmonella* agglutinating serum containing antibodies for all known *Salmonella* antigenic factors can be employed (authors' unpublished data Edwards¹²). When characteristic growth is obtained on SS Agar, or on the differential sugar mediums, it may be tested directly in a spot test with the polyvalent antiserum. A positive reaction however, is only presumptive evidence because numerous microorganisms of the enteric group possess antigens in common with the delineated genus *Salmonella* and a broader serologic and biochemical study of the culture undergoing the test might not warrant its recognition as a *Salmonella* type. A negative reaction would be presumptive evidence that the culture did not belong to the genus. This can be confirmed by noting its biochemical behavior.

A polyvalent anti-*Salmonella* agglutinating serum was prepared by members of the Veterinary Section of the 15th Medical General Laboratory by injecting a single rabbit with a suspension of all the known *Salmonella* antigens. This antiserum was distributed to numerous Army laboratories and was also employed by members of this installation. All of the *Salmonella* cultures which were classified serologically were agglutinated by the polyvalent serum. The fact that members of the genus *Salmonella* possess antigens in common with certain other members of the enteric group will lead to a number of false positive results. These are eliminated by complete serologic typing and a thorough biochemical analysis. For instance, *Shigella paradysenteriae* (Flexner I and II) possess somatic antigens in common with *Salmonella poona* and *Salmonella worthington* and a polyvalent *Salmonella* antiserum will agglutinate those *Shigella* types, yet further study readily separates them into their respective genera.

From the above discussion, it is concluded that *Salmonella* types ordinarily are hydrogen sulfide-positive and indol-negative. They ferment glucose but do not ferment lactose, salicin, or sucrose. They can be agglutinated on a spot plate with polyvalent anti-*Salmonella* serum, and cultures can be sent to laboratories which possess a complete *Salmonella* typing kit, for confirmation and classification.

SHIGELLA

In the same twenty-one month period referred to previously, 1121 cultures from feces were submitted to the Bacteriology Section for confirmation as shigellae. This figure includes only cultures submitted from United States Army Hospitals and Medical Laboratories, and is exclusive of cultures isolated in the 15th Medical General Laboratory. Of these, 735 (65 per cent) were *Shigella* types and 386 (35 per cent) were paracolon, *Proteus*, coliforms, and other bacteria. The application of certain tests, both serologic and biochemical, will reduce greatly the percentage of error and should aid laboratory workers in the selection of cultures to be submitted to other laboratories for typing.

While certain investigators may differ on one or two minor points, the following working definition of the genus *Shigella* (Borman, Stuart, and Wheeler¹) serves as a point of departure for this discussion: Members of *Shigella* are "aer-

obic, non-sporogenic, gram-negative, non-motile rods, anaerogenic in glucose (Infrequently, variants antigenically identical with normal forms are microaerogenic). Do not grow on bismuth sulfite agar. Uniformly do not form acetyl-methylcarbinol or H_2S , ferment lactose, utilize citrate, hydrolyse urea; very rarely reduce trimethylamine oxide. Inocula of young cells from broth cultures do not produce acid and usually do not grow in glucose broth at 45 C. Part of the antigens of certain varieties may be identical with those of the genus *Salmonella* but in no case are the major antigens of an S form of this genus identical with those of the Kauffmann-White schema. In nature, (organisms are) parasites of man."

In the general discussion, the treatment of cultures which appeared suspicious on Kligler's medium (modified) was carried to the urea medium stage; differentiation of *Shigella* cultures is continued from that point. Following the preliminary reading, those cultures which are found to be negative on the urea medium are tested in various *Shigella* antisera by the spot plate technic. A heavy suspension of the microorganisms may be made in formalinized physiologic saline solution from the growth on the Kligler's slants. Or, a plain infusion slant may be inoculated and incubated for about six hours and a suspension made from this. Cultures which produce blackening of modified Kligler's or Hajna's medium need not be tested in *Shigella* antisera. In the examination of hundreds of cultures of *Shigella* microorganisms, none was seen which produced blackening of the Kligler's medium. (See also the definition of the genus *Shigella* given by Borman, Stuart, and Wheeler.⁴)

Polyvalent and specifically adsorbed monovalent antisera are used to identify *Shigella* cultures. Methods for the preparation of such antisera are given by Boyd,⁵ Wheeler,²⁴ Weil *et al.*²³ Ewing,¹³ and others. Tests are first made in polyvalent and then in monovalent antisera. Proper use of antisera should enable the laboratory worker to classify tentatively nearly all of the dysentery types found in the Mediterranean area and those encountered in other localities as well.

A positive reaction in one of the polyvalent antisera is presumptive evidence that the culture is a *Shigella* type. Similarly, agglutination in one of the monovalent antisera is presumptive evidence that the culture is *Shigella paradysenteriae*, Flexner VI (*Shigella newcastle*, Boyd 88), *Shigella sonnei*, *Shigella ambigua*, etc.

Cultures which appear to be shigellae, but which agglutinate poorly, or not at all, should be tested for "heat-agglutinability". This may be done by heating a suspension of the bacterium in a water bath at 100 C. for thirty minutes. After such treatment, the suspension is retested for agglutination. The property of "heat-agglutinability" is noted particularly in *Shigella alkalescens* cultures,^{1,24} but is seen in other shigellae as well.^{3,23}

Frequently, it is desirable to obtain information quickly as to etiology in certain cases, or in a series of cases. If there are a number of nonlactose fermenting colonies on the original plates, spot agglutination tests may be made at that point, plain agar slants may be inoculated and spot tests made after about six hours' incubation. Presumptive information obtained in this way is often

of value in the control of an outbreak and of value to the clinician. Definitive biochemical tests and serologic studies, however, should never be omitted.

After the spot agglutination tests are completed, a culture should be confirmed as a member of the genus *Shigella* by a study of its biochemical and physiologic reactions, since it has been found that some paracolon bacteria cross-agglutinate in certain *Shigella* antisera.^{25,23} Furthermore, any culture which gives a typical reaction on Kligler's medium, is negative on urea medium, and fails to agglutinate in any of the *Shigella* antisera must be studied by means of biochemical tests. Such a culture may be one of the less common shigellae, a paracolon bacterium, or even a *Salmonella*.

The same list of biochemical and physiologic tests enumerated in the section on salmonellae, plus mannitol broth and Simmon's citrate agar, should be applied to microorganisms thought to be shigellae and to all cultures which do not agglutinate in *Shigella* antisera before they are submitted for further study and typing. Certain individual biochemical reactions will serve to eliminate most paracolons, aberrant coliforms, and the like (Table 1).

In routine work, we employ the oxalic acid paper method in *Shigella* studies, as well as in *Salmonella* cultures. While this method is slightly less sensitive than the methods which utilize Kovac's reagent, it is found that a high degree of correlation exists between the two methods. The indol reaction is an important tool in *Shigella* work and it can be relied on, since it is remarkably stable for the types. For definitive work within the genus *Shigella*, 1 per cent tryptone medium cultures and Kovac's reagent are recommended. The lead acetate paper is advised for *Salmonella* work and it must be remembered that the paper method is more sensitive than lead acetate agar, or Kligler's iron agar. *Shigella* types vary in their ability to blacken the lead acetate paper. This fact is stressed so that possible *Shigella* cultures will not be discarded if they are positive by this test.

Members of the genus *Shigella* are nonmotile. If a semisolid motility medium is employed many organisms which do not belong to the genus can be eliminated. Paracolon bacteria which might otherwise be confused with shigellae can often be eliminated from the latter group by this test alone.

All members of the genus produce acid from glucose. Organisms of the *Alcaligenes* group are found frequently in stool cultures and occasionally are submitted to us as *Shigella* types. *Alcaligenes fecalis* does not ferment glucose. It is sometimes confused with the shigellae because it produces an increased alkalinity on the slant in Kligler's medium; this gives the impression that acid has been produced in the butt. If an uninoculated tube of Kligler's medium is compared in such cases it will be observed that no color change has occurred in the butt of Kligler's medium. Similarly, cultures of *Pseudomonas aeruginosa* and related species produce increased alkalinity on the slant in Kligler's medium. In this case, there is usually a purplish cast to the medium caused by diffusion of pigment; the pigment is easily demonstrated by inoculating a plain agar slant or tube of broth. Furthermore, *Pseudomonas aeruginosa* cultures have a characteristic aromatic odor.

Mannitol is probably the most valuable carbohydrate used in *Shigella* work.

It is employed, not because it aids in the elimination of microorganisms from the genus *Shigella*, but because it serves to separate the *Sh. paradysenteriae* types, *Shigella alkalescens*, *Sh. sonnei*, *Sh. dispar*, etc., from *Shigella dysenteriae* (Shiga), *Sh. ambigua*, and the other mannitol-negative types.^{19,25} All *Sh. paradysenteriae* types thus far described ferment mannitol with the exception of certain cultures of *Sh. paradysenteriae*, Flexner VI (*Sh. newcastle*, Boyd 88).

Delayed fermentation (forty-eight hours or longer) of lactose by *Sh. sonnei* and *Sh. dispar* serve to separate these types from other shigellae. Only one in a series of fifty freshly isolated *Sh. sonnei* cultures produced acid within twenty-four hours; the others required from forty-eight hours to ten days. If the lactose tubes are plugged with corks dipped in paraffin after twenty-four hours' incubation, acid production by both *Sh. sonnei* and *Sh. dispar* tends to be accelerated. Early fermentation of lactose generally means that a microorganism is not a *Shigella* type, especially if gas is produced also. Paracolon bacteria are, by definition, late lactose fermenters. Most paracolon cultures produce relatively large amounts of gas from fermentable carbohydrates, but some are anaerogenic.^{21,25,26} Serologic methods, as well as additional biochemical and physiologic tests, will serve to differentiate both aerogenic and anaerogenic paracolon strains from members of the genus *Shigella*. It should be noted that if fermentation tubes are to be plugged, dye which is not an indicator of oxidation-reduction must be employed.

Sh. sonnei and *Sh. dispar* vary in their ability to produce acid from sucrose. Some *Sh. sonnei* cultures which were negative after forty days' incubation unplugged, produced acid in from seven to nine days in corked tubes. Other *Sh. sonnei* cultures were found to be negative at the end of sixty-one days in plugged sucrose tubes. Occasional cultures of *Sh. dispar* are encountered which ferment sucrose early, but most strains which produce acid require several days to do so. Many are negative in sucrose broth.

Occasionally a strain of *Sh. ambigua* is encountered which produces acid in sucrose (delayed). These can be differentiated easily by their inability to ferment mannitol and agglutination in specific serum.

Shigella cultures which are capable of fermenting sucrose are anaerogenic. Paracolon bacteria, while variable in their action on sucrose, are usually aerogenic. Anaerogenic paracolon cultures, regardless of their action toward this carbohydrate, can be differentiated from the shigellae on the basis of other biochemical tests, motility and serology. Motile anaerogenic paracolon bacteria are described by Stuart *et al.*²¹ and others.^{25,26}

Shigellae do not ferment salicin and, when this carbohydrate is available, it is quite useful, but as a rule it is not essential for separation of paracolon bacteria from *Shigella* cultures. All paracolon types do not ferment salicin but cultures which do, can be eliminated from both the genus *Salmonella* and the genus *Shigella*. As stated above, *Salmonella* cultures do not utilize adonitol and this substrate may be substituted for, or used in conjunction with, salicin. Adonitol is also of considerable value in *Shigella* work since, with the possible exception of *S. dysenteriae*,³ these microorganisms are not known to ferment it.

None of the described shigellae utilize citrate. For this reason, Simmon's

citrate agar is useful in separating those paracolons which utilize it. However, most *Salmonella* types utilize citrate, *S. typhi* and *Salmonella anatum* being notable exceptions.

Aerogenic variants of certain *Shigella* types may occur just as certain cultures of *Salmonella* may be anaerogenic. The original description⁸ of the serotype now known as *Sh. paradysenteriae*, Flexner VI, was a type which produced gas from fermentable carbohydrates and was mannitol-negative. This type was formerly known as the Newcastle bacillus and *Shigella newcastle*. Later, a variety which produced acid and gas from mannitol (Manchester type) was described.¹⁰ Boyd⁵ found anaerogenic cultures (Boyd 88 type) which were later shown to be serologically identical to the Newcastle and Manchester varieties. While these microorganisms generally are referred to as microaerogenic, others have been isolated in Africa and Italy which produce as much as 75 per cent of gas in fermentable carbohydrates (inside diameter of gas tube 4 to 5 mm.).

Boyd⁵ described microorganisms related to his P. 274 type (*Sh. paradysenteriae*, Boyd IV) which produce gas in fermentable carbohydrates (Wheeler *et al.*²⁵ also).

Aerogenic cultures related serologically to varieties⁶ of *Sh. dispar* are being studied in this laboratory.

Use of the above list of biochemical tests (Table 1) will enable laboratory workers to confirm cultures which do not agglutinate in antisera for the more common types as probable shigellae, to confirm cultures which agglutinate in the sera as shigellae, or as probable shigellae and, finally, to eliminate most paracolon cultures. There are exceptions in regard to fermentation of some carbohydrates so that it is impossible to make general statements which will cover all situations. Use of these notes, however, should increase greatly the number of confirmations of cultures submitted for specific typing.

SUMMARY

Biochemical and serologic tests are described which will aid laboratory workers in their identification of *Salmonella* and *Shigella* cultures. The methods outlined will serve to eliminate most microorganisms of doubtful significance in the laboratories in which they are first isolated.

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A NEW SELECTIVE MEDIUM FOR THE ISOLATION OF SALMONELLAE FROM STOOLS*

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Several selective plates were suggested for the isolation of pathogenic enteric microorganisms. Strongly selective mediums, such as the plate of Wilson and Blair¹⁰ and its modifications,^{1,8} the desoxycholate-citrate plate of Leifson⁵ and the brilliant green-lactose medium of Kauffmann³ are excellent for the isolation of *Salmonella* but do not permit the growth of many *Shigella* strains. They are used, therefore, in connection with less selective mediums on which all *Shigella* and fastidious *Salmonella* strains grow well, such as the desoxycholate agar of Leifson,⁵ the desoxycholate-lactose-sucrose medium of the Baltimore Biological Laboratory, the eosin-methylene blue agar,² and the bile salt plate of MacConkey.⁶ While larger or specialized laboratories use a series of different plates for each stool specimen, the small clinical laboratory in which fecal examinations are performed occasionally, may wish to use only one or two mediums. The S.S. (*Shigella-Salmonella*) agar of Difco, Inc. and the D.E.C. (Dysentery-Enteric-Cholera) plate of Panja and Ghosh⁷ can be used for this purpose. Both of these mediums offer certain advantages. The preparation of the D.E.C. plate, however, is time-consuming. The S.S. agar renders excellent service, but if it is used alone, some cases of infection will be missed.

The eosin-methylene blue agar (E.M.B.) is one of the best mediums for the isolation of fastidious shigellae and salmonellae, although it is nonselective. Various attempts have been made to make it a more selective medium suitable for the isolation of salmonellae if only few organisms are present. Knox *et al.*⁴ added brilliant green to this plate and secured a more selective effect. We attempted to utilize the inhibitory principle of sodium citrate and picric acid of the liquid enrichment medium for salmonellae devised by Ruys.⁹

Experiments with 87 *Salmonella* strains and their mixtures with *Proteus*, coliform and paracolon organisms have shown that the best selective action for salmonellae common in the United States is assured when 2 per cent sodium citrate and 2 per cent of a saturated watery solution of picric acid are added to the medium.

When the excellent qualities of the eosin-methylene blue agar as a medium for the isolation of fastidious enteric pathogenic organisms and the good results obtained with the same plate after the addition of sodium citrate and picric acid were taken into account, it seemed advisable to use them simultaneously for routine stools. The following procedure for the preparation of these two mediums, having the same base but a different selectivity, was found satisfactory for use in small laboratories.

1. Dissolve dehydrated E.M.B. agar, Difco, in the amount of water indicated

* Received for publication, September 9, 1946.

on the label of the bottle and add enough powdered agar to bring the concentration of agar to two per cent.

2. While hot, divide into two portions.

3. The first portion, without any further addition, is sterilized for twenty-five minutes at 12 to 13 lb. pressure. Pour into Petri dishes. This is the "original" eosin-methylene blue plate.

4. Add to the second portion two per cent of crystalline sodium citrate and sterilize as above.

5. Add to each 100 ml. of this portion two ml. of a concentrated watery solution of picric acid and 0.1 ml. of a 0.5 per cent watery solution of certified brilliant

TABLE 1
RESULTS OF STREAKING PLATES WITH MIXTURES OF PATHOGENIC AND NON-PATHOGENIC
ENTERIC MICROORGANISMS

PLATES USED	MIXTURE CONTAINING <i>E. coli</i> , <i>Ae. aerogenes</i> , <i>Pr. vulgaris</i> , <i>Ps. aeruginosa</i> and									TOTAL POSITIVE OF 450 PLATES	PROBABILITY OF POSITIVE RESULT, IN PER CENT
	<i>E. typhosa</i>	<i>S. paratyphi B</i>	<i>S. typhimurium</i>	<i>S. montevideo</i>	<i>S. pullorum</i>	<i>S. paratyphi A</i>	<i>Sh. paratyph. I</i>	<i>Sh. paratyph. II</i>	<i>Sh. sonnei</i>		
	of 50 experiments, positive result in:										
E.M.B.* original, alone	36	41	44	40	28	37	39	35	37	337	75
E.M.B. modification, alone	42	44	45	43	32	11	7	1	1	226	50
E.M.B. original and modi- fication, 1 plate each	44	45	47	43	40	39	40	35	37	370	82
E.M.B. original and modi- fication, 2 plates each	48	48	48	46	42	44	43	42	45	406	90
E.M.B. original and modi- fication, 3 plates each	48	49	48	48	47	46	46	48	48	428	95

* Eosin-methylene blue agar.

green. Pour into plates. This is the "modified" eosin-methylene blue medium.

Those who prefer not to use dehydrated products may prepare the base in the following way:

Dissolve 10 gm. peptone, 10 gm. lactose, 2 gm. crystalline dibasic potassium phosphate and 20 gm. agar in 900 ml. distilled water. Adjust pH to 7.2; add 40 ml. of a one per cent watery solution of Bacto-Eosin Y and 66 ml. of a 0.1 per cent aqueous solution of Difco-Methylene Blue. Then proceed beginning with step number 2, as above.

Inoculate the original plate lightly, the modified plate more heavily. Be careful that the surface of each plate is thoroughly dry. Read the plates after

twenty-four hours' incubation; the modified plate should be rechecked also after forty-eight hours.

Fifty series of plates were used for each experiment. Mixtures of coliform organisms, strains of *Proteus* and *Pseudomonas* with salmonellae and shigellae were streaked on the original E.M.B. agar and its modification. The mixtures were standardized in such a way that they contained approximately one *Shigella* or *Salmonella* for twenty-five organisms of each of the following species: *E. coli*, *Ae. aerogenes*, *Pr. vulgaris* and *Ps. aeruginosa*. The results grouped in Table 1 show that the use of one original E.M.B. plate and one modified E.M.B. plate gives a probability higher than 0.8 for the detection of the most frequent bacillary incitants of diarrhea. When multiple plates are used, e.g., duplicate sets, the probability of a positive result increases further. The method, therefore, seems to be useful for small laboratories.

SUMMARY

A modification of the eosin-methylene-blue plate, prepared by the addition of two per cent each of sodium citrate and a concentrated watery solution of picric acid, as well as 1:200,000 brilliant green, is recommended to be used simultaneously with the original eosin-methylene blue plate for the detection of pathogenic enteric microorganisms.

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INTRADERMAL REACTIONS IN TRICHINOSIS*

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During the latter part of February 1945, an epidemic of trichinosis occurred among a large group of German prisoners of war. Transmission of the disease resulted from the ingestion of improperly cooked pork. The ensuing infection was mild in character as judged by the acute symptoms which subsided within twenty days following admission to the hospital. Although more than 250 persons contracted trichinosis, there were no fatalities. In addition, an equal or greater number of prisoners in the camp ate infected meat, but did not develop symptoms of sufficient severity to warrant reporting to the dispensary. This epidemic offered an unusual opportunity to evaluate the clinical and immunologic aspects of trichinosis in a controlled group of patients. The present report includes the results of skin tests of infected, presumably infected, and uninfected individuals living under similar conditions. The epidemiologic and clinical data will be reported in detail elsewhere.

The diagnosis of trichinosis was based on typical clinical findings coupled with the presence of eosinophilia. Conclusive proof of the etiology was never obtained even though specimens of blood from 94 patients were unsuccessfully examined for larvae during the acute phase. In addition, duodenal washings from 8 patients and stool specimens in 64 instances failed to reveal the presence of adult trichinae. Muscle biopsies were made in 36 instances; encysted trichinae were found in only one case a year after the onset of illness. Clinically, the symptoms of circumorbital edema, generalized muscular aches and pains, sweating and weakness were characteristic. The initial total leukocyte counts and eosinophile levels are presented in Table 1. Although the former were not significantly elevated, the finding of eosinophilia in 88.7 per cent of the patients strongly supported the diagnosis.

THE RESULTS OF SKIN TESTS

Since all prisoners were under rigid military discipline, it was possible to obtain specimens and to conduct laboratory tests within the first few days of symptoms. Fortunately, within twenty-four hours after the identity of the disease was established, sufficient trichina extract to conduct large scale skin testing was made available. The tests were performed by injecting 0.05 ml. of antigen, diluted 1:10,000, into the epidermis of the flexor surface of the forearm. The resultant wheal was compared in size with a saline control after fifteen minutes and again at the end of twenty-four hours. Immediate or delayed reactions ranging in diameter from 6 to 8 mm. were considered doubtful; those wheals

* Received for publication, August 23, 1946.

which exceeded 8 mm. were called positive. The patients were retested at the end of three, six and twelve weeks after the onset of symptoms. The summarized data are presented in Table 2. Antigens A, B and C were prepared according to the method of Bozicevich.⁷ Antigen D represented a phosphate buffer extract of dried trichina larvae preserved with 1:10,000 merthiolate. Antigen E was a saline extract which had been placed in boiling water for fifteen minutes after the method of Witebsky *et al.*²⁵ Antigen F was prepared by the original method of Bachman¹ in which C'oca's fluid was utilized as the extracting medium.

Tests with antigen A during the initial stages of the disease revealed 8 per cent positive immediate and 17 per cent positive delayed reactions. Three weeks later, 211 patients were retested with antigen B, which showed an incidence of only 5 per cent immediate and 4 per cent delayed reactions. Since an increase in sensitivity rather than a decrease was expected, 32 patients were simultaneously examined with five different antigens, as shown in Table 2. Surprisingly

TABLE 1

INITIAL LEUKOCYTE COUNTS AND EOSINOPHILE LEVELS IN 212 PERSONS WITH TRICHINOSIS

NUMBER OF LEUKOCYTES IN THOUSANDS	PER CENT OF CASES	PERCENTAGES OF EOSINOPHILES	PER CENT OF CASES
3.1 to 5	10.3	0 to 5	11.3
5.1 to 6	20.7	6 to 10	21.7
6.1 to 7	22.2	11 to 20	39.2
7.1 to 8	20.0	21 to 30	19.3
8.1 to 9	8.0	31 to 40	4.2
9.1 to 10	6.1	41 to 50	3.3
10.1 to 12	9.4	51 to 60	0.5
12.1 to 14	3.2	61 to 70	0.5

enough, variations from 5 to 50 per cent immediate type reactions were encountered in the same patients. Similar tests carried out on a larger group at the six week interval revealed the same discrepancy in skin response to different antigens. It is of interest to note that the highest value obtained by the immediate type of intradermal reaction was only 40 to 50 per cent. If the doubtful cases were included, a maximal percentage of positivity ranged from 61 to 75. Standard antigens, such as B and D, produced significant wheals in only 6 and 18 per cent of the cases at the six week interval, levels which do not coincide with those reported by other investigators. It should also be noted that the delayed reactions, which supposedly occur only during the acute phase of trichinosis, progressively increased with antigen E from 9 to 18 to 28 per cent over the test period.

During the time of infection, a large number of prisoners had eaten uncooked pork but, nevertheless, remained free of symptoms. Groups of these individuals were skin-tested three, six and twelve weeks after the onset of illness with the results summarized in Table 3. The first 50 such individuals, who were examined

with four different antigens, showed variations from 8 to 28 per cent of immediate reactions and from 8 to 20 per cent of delayed reactions. Again antigen E was most effective and preparation B elicited more skin responses in the above

TABLE 2

INTRADERMAL TESTS IN INFECTED AND HOSPITALIZED PATIENTS WITH TRICHINOSIS

TIME OF TESTING IN RELATION TO ONSET OF DISEASE	TRICHINA ANTIGEN USED, DILUTION 1:10,000	IMMEDIATE TYPE OF REACTION			DELAYED TYPE OF REACTION		
		Number of persons tested	Per cent doubtful	Per cent positive	Number of persons tested	Per cent doubtful	Per cent Positive
Onset	A	249	5	8	249	14	17
	A	32	22	15	32	3	0
	B	243	9	5	243	5	4
3 weeks after onset	C	32	53	9	32	0	0
	D	32	47	19	32	19	3
	E	32	25	50	32	19	9
6 weeks after onset	B	108	19	6	108	7	7
	D	240	15	18	226	13	10
	E	240	18	42	226	12	18
	F	108	9	17	108	5	12
12 weeks after onset	E	112	15	46	104	19	28
	F	112	17	38	104	24	26

TABLE 3

INTRADERMAL TESTS IN ASYMPTOMATIC INDIVIDUALS AFFECTED WITH TRICHINAE

TIME OF TESTING AFTER ONSET OF EPIDEMIC	TRICHINA ANTIGEN USED, DILUTION 1:10,000	IMMEDIATE TYPE OF REACTION			DELAYED TYPE OF REACTION		
		Number of persons tested	Per cent doubtful	Per cent positive	Number of persons tested	Per cent doubtful	Per cent positive
3 weeks	B	50	12	10	50	14	10
	D	50	12	8	50	16	8
	E	50	22	28	50	16	20
	F	50	18	10	50	16	16
6 weeks	B	78	3	8	64	19	8
	D	78	4	9	64	21	6
	E	78	6	15	64	12	15
	F	78	3	15	64	10	15
12 weeks	E	55	16	25	55	7	20
	F	55	15	18	55	2	22

persons than in hospitalized patients (compare with Table 2). A second group of 78 individuals,* selected at random from those who ate infected meat, showed

* Thirty-one of these subjects were originally tested at the three week interim.

a lower incidence of positive skin tests after six weeks. At the end of twelve weeks, however, there was a relative increase in number of immediate type reactions with antigens F and E, the percentage of reactions ranging from 18 to 25. It should again be noted that a significant number of delayed reactions were obtained throughout all test periods. The diagnosis of trichinosis in the

TABLE 4
INTRADERMAL TESTS IN UNINFECTED CONTROLS

TIME OF TESTING AFTER ONSET OF EPIDEMIC	TRICHINA ANTIGEN USED, DILUTION 1:10,000	IMMEDIATE TYPE OF REACTION			DELAYED TYPE OF REACTION		
		Number of persons tested	Per cent doubtful	Per cent positive	Number of persons tested	Per cent doubtful	Per cent positive
Onset	A	27*	4	0	27*	4	0
3 weeks	B	35	3	3	35	3	0
	D	35	3	3	35	0	0
	E	35	3	3	35	0	0
	F	35	6	3	35	3	0
12 weeks	E	54	6	6	54	6	2
	F	54	4	4	54	7	2

* Americans who were hospitalized.

TABLE 5
DETECTION OF TRICHINOSIS BY MEANS OF DELAYED SKIN REACTIONS

TIME OF TESTING AFTER ONSET OF EPIDEMIC	TRICHINA ANTIGEN USED, DILUTION 1:10,000	TOTAL NUMBER OF PERSONS TESTED	DELAYED REACTIONS POSITIVE			PERCENTAGE OF PATIENTS WITH POSITIVE REACTIONS	
			Immediate Reaction		Total	Delayed	Immediate (See Table 2)
			Doubtful	Negative			
Onset	A	249	5	0	5	2	8
3 weeks	B	243	2	7	9	3	5
	E	32	1	0	1	3	50
6 weeks	E	226	9	11	20	9	42
12 weeks	E	110	4	13	17	15	46

asymptomatic prisoners was strengthened by the finding of eosinophilia in over 70 per cent within three weeks after the time of presumed infection.

It was also unusual that during the epidemic period, no cases of trichinosis occurred among persons in the camp who ate at one particular mess. In order to rule out previous infection in the prison population as a whole and to obtain comparable controls, intradermal tests were performed on these individuals. The results are presented in Table 4. The data indicate that the immediate and delayed reactions which were observed among the hospitalized and asymptomatic

prisoners represented specific responses to trichinous infection. It was not entirely possible to rule out infection among the controls because practically all admitted a fondness for pork sausage cooked in the manner which had resulted in the epidemic herein described. The majority had eaten similar meat on numerous occasions prior to their detention as prisoners of war. The eosinophile counts on the first 50 controls ranged within normal limits.

The observation that the intradermal test as a means of diagnosing mild trichinosis was less than 50 per cent accurate led to a reexamination of the data for the purpose of increasing sensitivity by combining both the immediate and delayed reactions. The results are shown in Table 5. By adding the cases detected by delayed reactions alone, the over-all sensitivity was increased by as much as 15 per cent in the group tested twelve weeks after infection. Furthermore, among 55 asymptomatic but infected patients studied at the same time, 6 additional ones could be selected by the delayed skin test, thus elevating the sensitivity 11 per cent.

The data were also reviewed for the constancy of the reaction in the same patients subsequently tested, six and twelve weeks after onset of illness. Comparative studies in 103 patients, using antigen E, revealed that identical results, *i.e.*, positive or negative immediate tests, were obtained in 63 per cent of the patients on both occasions. In 15 per cent, the skin test was reversed from positive at six weeks to negative at twelve weeks, with the same antigen. The remaining 22 per cent were classed as nonreactors at six weeks, but showed skin tests which became positive after twelve weeks. Such data are difficult to interpret since they add complicating host factors to the already complex problem of antigen preparation.

DISCUSSION

As a result of a number of key studies on trichinosis, the skin test has gradually been accepted as one of the most reliable diagnostic aids in this disease.^{3, 6, 7, 9, 13, 15, 16, 17, 18, 19, 21, 22, 27} The usual procedure has involved intradermal testing with 1:10,000 antigen in an attempt to produce the characteristic blanched and elevated wheal within a period of ten to fifteen minutes following injection. An interpretation of the result is not at all difficult when the reaction is typical, or the reading is negative, provided adequate controls are used. There are, unfortunately, a number of borderline reactions which may be variously recorded by different individuals. Another difficulty is the fact that no standard size of wheal has been established or generally accepted by all investigators as a criterion of a positive reaction. Further weaknesses in the utilization of skin tests have been the lack of repeated examinations with the same antigen on large groups of individuals over a period of time and also the failure to standardize antigen preparations and test dosage. In the group of cases herein described, the pronounced variations in the degree of skin reaction to different lots of the same antigen, or to extracts prepared by other methods, were too striking to be ignored. Limited observations of the same nature have been made by other investigators.^{1, 11} This fluctuation may not necessarily be caused by the

method of preparing the antigen, as we encountered it with three different lots of material handled in a similar manner. On the other hand, Antigen E was so markedly superior to the others that it merits special discussion. It was originally introduced by Witebsky, Wels and Heide²⁵ as a complement-fixing agent. Its effectiveness in skin tests was subsequently reported by Arbesman, Witebsky and Osgood.¹ They found no apparent difference in the size of the reactions when compared with unboiled antigen in 28 uninfected allergic patients. The antigen E utilized in these studies was furnished by Dr. Witebsky who informed us that he had found it highly satisfactory for skin tests.²⁵ Aside from the above observations, this particular type of antigen had not heretofore been utilized for intradermal tests. Basically, it represented the supernate of an alkaline saline extract of dried and powdered larvae which had been placed in a bath of boiling water for fifteen minutes. The precipitate which formed was removed by centrifugation and the clear supernate was utilized.²⁵ Our skin test results with the most highly reactive agent and even by including the delayed positive responses did not exceed a maximum diagnostic level of 61 per cent. Furthermore, if antigens of the type generally used at present (B, C and D) had been the only ones available, the degree of skin response during the first six weeks following infection would not have exceeded 19 per cent. Such results may be contrasted with those obtained by others in which positive reactions varying from 70 to more than 90 per cent have been reported.^{2, 6, 13, 16, 19, 22, 24}

The above differences possibly may have been owing to the fact that the epidemic in the camp was extremely mild and that universal sensitivity to trichina larvae did not develop. Such an explanation would be at variance with statements that the reactivity of the skin bears no relationship to the severity of the disease.^{7, 11, 13, 19, 21, 23} This concept is largely based on the failure to elicit skin responses in moribund patients and, in one instance,¹¹ upon the lack of correlation between reactions during life and the number of larvae per gram of muscle found at necropsy. On the other hand, the positive relationship between the degree of infection and the skin response, herein suggested, is supported by the fact that persons with asymptomatic trichinosis exhibited a degree of skin reaction intermediate between that of hospitalized patients and normal controls. Thus, with antigen E, the symptomatic cases showed immediate reactions totaling 50 per cent, whereas the asymptomatic group were positive in only 28 per cent of instances. Similar differences were also obtained at the end of six and twelve weeks. A second point in harmony with this concept is the observation that among 35 of the most severely ill patients in the epidemic, 74 per cent showed positive and 23 per cent exhibited doubtful intradermal reactions at one or more of the test intervals. These data are supported by the related findings of a few other workers.^{8, 20}

Intradermal tests of the same individuals with multiple antigens were undertaken with a full realization of the possibilities of inducing artificial sensitivity by this procedure.^{1, 5, 10} Not all authors, however, agree that active immunization to repeated injection of antigen occurs with any degree of frequency.^{14, 19} The data presented in Table 3 do not favor the concept of artificial active sensi-

zation in that no significant rise in the percentage of positive skin reactors was encountered. Potential false positive reactions resulting from ascaris or trichiuris infestation were ruled out by the examination of stools from 135 patients. These parasites were not present in any of the specimens submitted.

SUMMARY

A group of more than 200 individuals with trichinosis was examined by means of intradermal tests at intervals of three, six and twelve weeks following the onset of acute symptoms. The epidemic was mild in character and no deaths resulted. Positive immediate skin responses were obtained in from 42 to 50 per cent of cases using an alkaline saline extract of trichina larvae which had been placed in boiling water to remove heat precipitable material. Antigens prepared by the methods commonly employed differed markedly in activity from each other and lacked sensitivity. A maximal value of only 38 per cent for immediate reactions was obtained with one of these extracts whereas the remaining preparations ranged in sensitivity from 5 to 19 per cent. Delayed skin reactions with all antigens gradually increased in number during each period of testing and, after twelve weeks, were positive in from 26 to 28 per cent of cases. These reactions were found to be specific for trichinosis and when combined with the immediate ones increased the sensitivity of the skin test from 2 to 15 per cent.

A second group of 152 persons, presumably infected but without symptoms, was also tested with the same trichina extracts. The maximal value for immediate positive wheals was 28 per cent with the heated antigen and variations ranging from 8 to 18 per cent were noted with the others. A total of 89 uninfected individuals from the same environment, who were utilized as controls, showed an incidence of positive immediate tests from 0 to 6 per cent with all antigens.

As a result of experience gained in this epidemic, it is concluded that the skin reaction is a reliable procedure for the diagnosis of trichinosis, provided a typical wheal is produced. The extreme variability of different lots of trichina extract in the same individuals points to the necessity of introducing a standard preparation of constant composition and reactivity for skin testing purposes. The proper concentration of such an antigen which would be required to induce maximal, specific skin responses remains to be investigated.

Acknowledgments. The following individuals and institutions furnished us with liberal supplies of trichina larvae and extracts, thus making this study possible: Dr. S. E. Gould, Wayne County General Hospital, Eloise, Michigan; Dr. E. Witebsky, University of Buffalo School of Medicine; Dr. H. D. Piersma, director, Human Biological Division, Lederle Laboratories; Dr. J. H. Sandground, Lilly Research Laboratories, Eli Lilly and Company; and Dr. F. J. Brady, acting chief, Zoology Laboratory, U. S. Public Health Service. The authors also wish to express their gratitude to Brigadier General Joseph E. Bastion for his encouragement and interest in this work and for his efforts in making available facilities of the Percy Jones Hospital Center. The following officer and enlisted men furnished invaluable voluntary technical assistance: C. Becker, 1st Lt., SnC.; A. S. Resnick, T/Sgt.; R. Kramer, T/4; O. Bower, Pfc.; C. Childs, T/4; S. Kennedy, T/5; and R. Brotherton, Pfc.

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COMPLEMENT-FIXATION AND PRECIPITIN TESTS IN TRICHINOSIS*

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An outbreak of trichinosis among a large group of prisoners of war presented an unusual opportunity to study the immunologic aspects of this disease under relatively controlled conditions. The clinical report and the results of intradermal tests have been presented elsewhere^{1, 3}. The present investigation concerns the findings with complement-fixation and with precipitin tests utilizing serums obtained at the onset of symptoms, and three, six and twelve weeks later.

METHODS

Complement-fixation tests were performed according to the method of Witebsky, Wels, and Heide,⁴ using 1:2000 antigen supplied by them.† The serums were inactivated for thirty minutes in a 56 C. water bath. The initial examination consisted of a three tube screening test performed as follows. Two tubes each received 0.1 ml. of 1:5 serum and a third tube, 0.1 ml. of 1:10 serum. Trichina antigen (0.1 ml. of a 1:2000 dilution) was added to tubes one and three and an equal amount of saline was delivered to tube two. All tubes then received 0.1 ml. of 1:15 or 1:20 lyophilized complement. The tests were held at 4 C. overnight and incubated for thirty minutes at 37 C. In the morning, amboceptor was titrated as follows. Dilutions of amboceptor consisting of 1:500, 1:750, 1:1000, 1:1500, 1:2000, 1:3000, 1:4000 and 1:6000 were prepared and 0.6 ml. of each dilution was placed in separate tubes. Complement, 1:15 or 1:20 incubated at 4 C. overnight and for thirty minutes at 37 C., was delivered to each of the above eight tubes in the amount of 0.2 ml. Each tube also received 0.2 ml. of 5 per cent washed sheep cells. The mixtures were shaken and incubated in the water bath at 37 C. for thirty minutes. One unit of amboceptor was considered to be the smallest amount which gave complete hemolysis in thirty minutes. For the actual test, 3 units of amboceptor were used to sensitize an equal volume of 5 per cent washed red cells; 0.2 ml. of this mixture was delivered to each tube in the test. Known positive and negative serums were included together with an antigen control. Those serums which showed complete fixation of complement in a dilution of 1:10 were titrated to the endpoint the following day.

The precipitin tests were conducted in small serologic tubes as follows: 0.05

* Received for publication, August 23, 1946.

† Miss Heide was able to spend a few days in our laboratory, which insured that the two techniques were comparable.

ml. of unactivated serum was added to each of two tubes. The first was overlaid with 0.05 ml. of 1:500 antigen and the second received extracting fluid alone as a control. Readings were made after incubation for one and two hours at room temperature. The appearance of a fine white precipitate at the junction of the serum and the antigen was considered as evidence of a positive reaction.

RESULTS WITH COMPLEMENT FIXATION

The data obtained from serums of infected patients at four different intervals are presented in Table 1. Of 248 specimens examined during the acute phase, when the disease was first detected, none showed positive fixation and two

TABLE 1
COMPLEMENT-FIXATION TESTS IN INFECTED AND HOSPITALIZED PATIENTS WITH TRICHINOSIS

TIME OF TESTS AFTER ONSET OF ILLNESS	NUMBER OF PERSONS TESTED	PER CENT POSITIVE	PER CENT DOUBTFUL
Onset	248	0	1
3 weeks	249	37	15
6 weeks	239	34	7
12 weeks	120	36	8

TABLE 2
COMPLEMENT-FIXATION TITRATIONS IN INFECTED AND HOSPITALIZED PATIENTS WITH TRICHINOSIS

TIME OF TEST AFTER ONSET OF ILLNESS	NUMBER OF PERSONS TESTED	PER CENT OF PATIENTS SHOWING TITERS OF		
		1:20	1:40	1:80
3 weeks	80	13	13	15
6 weeks	80	15	8	1
12 weeks	43	10	2	0

(1 per cent) gave doubtful results. Three weeks later, the number of positive results rose to a maximum of 37 per cent and remained at a relatively constant level during the remaining test periods. It might be argued that with an increase in time interval between infection and sampling, *i.e.*, six months, the complement-fixation test would have attained a greater degree of sensitivity. Such an assumption does not fit in with the titers of positive serums presented in Table 2. At three weeks, 28 per cent of serums showed definite fixation of complement in dilutions of 1:40 and 1:80; nine weeks later, only 2 per cent maintained similar antibody titers.

As was previously pointed out,¹ two additional groups were also tested serologically. The first included a number of individuals who had eaten uncooked pork but remained free of symptoms (infected asymptomatic). The second group consisted of prisoners who had eaten in a particular mess where all food

was thoroughly cooked and among whom no cases of trichinosis occurred (uninfected controls). The results of complement-fixation tests on sample populations from these two groups with serums obtained at the same intervals are presented in Table 3. Among those presumably infected but asymptomatic, serologic positivity ranged from 4 to 12 per cent. By contrast, the uninfected controls evidenced negative serologic reactions on two separate occasions. These data are in accord with the results of intradermal tests,¹ in which it was also found that a number of individuals showed strongly positive reactions, although they did not manifest symptoms of trichinosis.

The constancies of the complement-fixation tests were compared using serums

TABLE 3
COMPLEMENT-FIXATION TESTS IN INFECTED ASYMPTOMATIC PERSONS AND IN UNINFECTED PERSONS

TIME OF TEST AFTER ONSET OF EPIDEMIC	INFECTED ASYMPTOMATIC			UNINFECTED CONTROLS		
	Number of persons tested	Per cent positive	Per cent doubtful	Number of persons tested	Per cent positive	Per cent doubtful
3 weeks	50	12	0	55	0	0
6 weeks	77	4	4			
12 weeks	55	11	2	54	0	4

TABLE 4
COMPLEMENT-FIXATION TESTS FOR TRICHINOSIS TO SHOW CONSTANCY OF RESULTS IN TWO CONSECUTIVE SAMPLES

RESULT OF TEST IN INTERVALS AFTER OUTBREAK		NUMBER OF PERSONS TESTED	PER CENT OF TOTAL
3 weeks	6 weeks		
Neg.	Neg.	130	61.3
Pos.	Pos.	58	27.3
Neg.	Pos.	17	8.0
Pos.	Neg.	7	3.3
Totals.....		212	99.9

obtained at three weeks and again at six weeks following symptoms (Table 4). For purposes of simplicity, all doubtful reactions were considered as negative. It should be noted that, in 88.6 per cent of instances, duplicate results were in agreement with each other in that either consistently negative or positive findings were obtained. In 8 per cent of the cases previously negative, serology had become positive during the three week interval. An additional seven patients (3.3 per cent) showed reversal of serologic findings, either as a result of error in technic, or owing to a loss in antibody content. That the latter explanation is the more likely cause of the reversal is suggested by the fact that doubtful reactions were observed in four of the seven cases at the six week period.

The precipitin tests, in our hands, proved to be considerably less sensitive

than either the complement-fixation or intradermal procedures. Using a single antigen dilution of 1:500, a maximum value of 6 per cent positive results was obtained twelve weeks after the initial symptoms of the disease (Table 5). The cases of asymptomatic or presumed infection together with the uninfected controls have also been included. Antigen F was prepared by the method of Bachman using Coca fluid as the extractive. Antigen E was obtained from Doctor Witebsky, utilizing an alkaline saline extraction followed by heating in a water bath.⁴ On the whole, the results of the precipitin tests were difficult to interpret. Some normal serums showed a hazy precipitate with the trichina antigen at the interphase. Such reactions were not readily distinguishable from the supposedly specific ones which appeared when serums from infected cases were tested. Attempts to emphasize the reaction by increasing or decreasing

TABLE 5
PRECIPITIN TESTS IN PERSONS WITH SYMPTOMATIC AND ASYMPTOMATIC INFECTIONS
AND IN UNINFECTED PERSONS

TIME OF TEST AFTER OUTBREAK OF EPIDEMIC	ANTIGEN 1:500	NUMBER OF PERSONS TESTED	STATUS OF INFECTION	NUMBER OF TESTS POSITIVE	PER CENT POSITIVE
Onset	F	250	Symptomatic	2	1
3 weeks	F	248	Symptomatic	13	5
	F	50	Asymptomatic	2	4
	F	35	Uninfected	0	0
6 weeks	F	222	Symptomatic	2	1
	E	222	Symptomatic	0	0
	F	77	Asymptomatic	0	0
	E	77	Asymptomatic	0	0
12 weeks	F	120	Symptomatic	7	6
	F	55	Asymptomatic	1	2
	F	54	Uninfected	0	0

the concentration of antigen were unsuccessful. Furthermore, we were unable to obtain reduplicable tests with the same serums on different occasions in the hands of the same or another individual making the examination. As a result of these discrepancies positive reactions were assigned only to those specimens which yielded a definite ring containing a fine precipitate at the interphase of serum and antigen. The above problem remains to be investigated further.

DISCUSSION

From the data herein presented, one would be forced to conclude that the complement-fixation test is sufficiently sensitive to detect a maximum of only 37 per cent of symptomatic and 12 per cent of asymptomatic cases of trichinosis. The procedure, however, must be evaluated in the light of the fact that the

epidemic was extremely mild in character and that the present report is limited to the technic originated by Witebsky, Wels, and Heide.⁴ The fact that discrepant results were obtained on duplicate specimens of serum in only 3.3 per cent of the specimens tested is encouraging and suggests that the procedure is reliable and amenable to improvements in technic.

On the basis of studies conducted during the epidemic, it is now realized that all of the diagnostic tests which were employed lacked sensitivity. In fact, some preparations for intradermal testing were active in only from 5 to 19 per cent of cases.¹ It would appear from our experience that serologic procedures need to be completely re-evaluated and standardized if they are to be of value in the diagnosis of trichinosis. An approach to this problem is presented elsewhere.²

SUMMARY

More than 200 serums from patients with trichinosis were studied by means of a complement-fixation test at intervals of three, six and twelve weeks following the onset of acute symptoms. The maximal incidence of positive results with this procedure was 37 per cent at the three week period. The incidence of positive complement-fixation reactions varied from 4 to 12 per cent in a second group of presumably infected but asymptomatic cases. Comparable serologic checks were obtained from the same patients tested at two different intervals. Similar studies with the precipitin reaction yielded an incidence of from 0 to 6 per cent positive reactions. This procedure was not considered to be reliable or reduplicable. As a result of these studies, it is suggested that all serologic diagnostic tests in trichinosis be reviewed for the purpose of introducing standard methods of increased sensitivity.

Acknowledgments. We are indebted to Dr. E. Witebsky and to Miss Anne Heide for generous supplies of antigen and actual assistance in performance of the tests. Dr. J. H. Sandground of Eli Lilly & Company and Dr. H. D. Piersma of Lederle Laboratories kindly furnished additional antigens for precipitin tests. The authors also wish to express their gratitude to Brigadier General Joseph E. Bastion for his encouragement and interest in the problems involved. The following officer and enlisted men furnished invaluable voluntary technical assistance: C. Becker, 1st Lt., Sn.C.; A. S. Resnick, T/Sgt.; R. Kramer, T/4; L. Bower, Pfc.; C. Childs, T/4; S. Kennedy, T/5; and R. Brotherton, Pfc.

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IMMUNOLOGIC REACTIONS IN TRICHINOSIS WITH PURIFIED ANTIGENS*

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In 1942, Melcher isolated two serologically active fractions from *Trichinella spiralis* larvae, an acid-soluble protein and a polysaccharide. The former consisted of three electrophoretic components and elicited intradermal and precipitin reactions in rabbits; the latter showed precipitin activity only.⁴ For the purpose of testing these substances in human beings Dr. Melcher sent us lyophilized samples of the acid-soluble protein, an alkaline extract which presumably contained both protein and polysaccharide, and defatted *Trichinella* larvae. The polysaccharide was prepared in our laboratory with strict adherence to the method described by Melcher.⁴ The above materials were then utilized for intradermal, precipitin, and complement-fixation tests on 120 individuals who had contracted trichinosis three months previously. The methods of conducting the tests have been given in detail elsewhere.^{2,3}

INTRADERMAL REACTIONS

A total of six antigens and three control fluids were used for the intradermal test. The partly purified antigens were carefully weighed, diluted with distilled water to make 1:5000 solutions, and centrifuged at high speed until they proved to be bacteriologically sterile. The results with antigens E and F have been reported previously.² The former was a 1:10,000 dilution of a heated extract prepared by the method of Witebsky, Wels, and Heide;⁷ the latter was a 1:10,000 dilution of larvae extracted with Coca's solution. The control fluids were treated in a manner identical with the extracts except that larvae were omitted. Patients who reacted to the control material were not included in the findings.

The data in Table I reveal that the procedure utilized for purification of trichina antigens did not serve to increase the concentration of the reactive intradermal ingredient or ingredients in the various preparations. This is manifest by the fact that the purified fractions produced fewer positive wheals, although they were more than twice as concentrated as antigens E and F. The latter were diluted to 1:10,000 on the basis of the total weight of defatted larvae originally added. Actually, the amount of active principle present must have been far less. The saline extract, which was the least purified of all fractions, elicited 25 per cent immediate and 33 per cent delayed positive skin responses. The acid-soluble protein was distinctly less reactive and the polysaccharide proved to be practically inert.

* Received for publication, August 23, 1946.

In Table 2 the results of intradermal tests are presented with 55 asymptomatic persons, who were presumably infected with trichinosis. The incidence of positive immediate and delayed skin responses in the group was somewhat lower than among the patients who acknowledged symptoms. The order of activity of the various antigens, with the exception of the polysaccharide, did not vary significantly. The above antigens were also tested in 54 persons from a company in which no cases of trichinosis had occurred.^{5, 2} The incidence of positive results in this group varied from 4 to 6 per cent for immediate positive reactions and from 0 to 2 per cent for the delayed type of wheal. From these data,

TABLE 1
INTRADERMAL TESTS WITH PURIFIED ANTIGENS IN INFECTED AND HOSPITALIZED
PATIENTS WITH TRICHINOSIS

ANTIGEN	NUMBER OF PERSONS TESTED	IMMEDIATE TYPE OF REACTION		NUMBER OF PERSONS TESTED	DELAYED TYPE OF REACTION	
		Per cent doubtful	Per cent positive		Per cent doubtful	Per cent positive
E	112	15	46	104	19	28
F		17	38		24	26
Saline extract		19	25		23	33
Acid-soluble protein		15	18		17	29
Polysaccharide		17	8		6	9

TABLE 2
INTRADERMAL TESTS WITH PURIFIED ANTIGENS IN 55 PERSONS ASYMPTOMATIC
INFECTED WITH TRICHINAE

ANTIGEN	IMMEDIATE TYPE OF REACTION		DELAYED TYPE OF REACTION	
	Per cent doubtful	Per cent positive	Per cent doubtful	Per cent positive
E	16	25	7	20
F	15	18	2	22
Saline extract	16	13	9	18
Acid-soluble protein	16	7	2	20
Polysaccharide	16	9	2	7

it would appear that the skin responses induced by the various fractions of trichina larvae represented specific antigen-antibody reactions.

PRECIPITIN TESTS

For the precipitin test, all antigens were diluted 1:500 in saline and examined by the method formerly described.³ As can be seen from table 3, none of the fractions proved to be more sensitive than antigens E and F. In order to eliminate the possibility of a zone phenomenon, twenty serums from hospitalized patients were tested with the various antigens up to dilutions of 1:320,000 with negative results. These findings are in accord with our previous experience with the precipitin test.⁵

COMPLEMENT-FIXATION TESTS

Specimens of serum obtained twelve weeks after the onset of symptoms were examined by means of the complement-fixation procedure originally described by Witebsky *et al.*⁷ The three purified fractions were first titrated against a single positive serum; a dilution of 1:1000 was then selected for the preliminary test against varying amounts of eight different serums. The titers with both weak and strong serums were practically identical for the acid-soluble protein, the saline extract, and the Witebsky antigen. The polysaccharide was completely inactive. A total of 120 serums in dilutions of 1:5 and 1:10 were then tested against the four different antigens with the results presented in Table 4. The incidence of positive complement-fixation reactions varied from 36 to 37 per cent with three of the antigens, whereas the polysaccharide again proved to

TABLE 3
PRECIPITIN TESTS FOR TRICHINOSIS WITH PURIFIED ANTIGENS

STATUS OF PERSON TESTED WITH RESPECT TO INFECTION	NUMBER OF PERSONS TESTED	PER CENT POSITIVE PRECIPITATES WITH DESIGNATED ANTIGEN				
		E	F	Saline extract	Acid protein	Polysac- charide
Symptomatic.....	120	6	5	1	2	0
Asymptomatic.....	55	0	0	2	2	0
Uninfected.....	54	0	0	0	0	0

TABLE 4
COMPLEMENT-FIXATION TESTS FOR TRICHINOSIS WITH PURIFIED ANTIGENS

STATUS OF PATIENT WITH RESPECT TO INFECTION	NUMBER OF PERSONS TESTED	PER CENT OF POSITIVE RESULTS WITH DESIGNATED ANTIGEN			
		E	Saline extract	Acid protein	Polysac- charide
Symptomatic.....	120	36	36	37	0
Asymptomatic.....	55	11	7	9	0
Uninfected.....	54	0	0	0	0

be inactive. The similarity of the results was attributable to the constancy with which a particular serum fixed complement with all three antigens.

The above data clearly indicate that the substance or substances responsible for the complement-fixation test were absent from the polysaccharide and present, but not concentrated, in the saline extract or in the acid-soluble protein. The fact that boiling was utilized by Witebsky, Wels, and Heide⁷ as a means of removing certain of the impurities from the original extract indicated that the antigenic components were heat-stable.⁶ Since the acid-soluble protein was the purest active substance at our disposal, the following experiment was performed. A 1:500 dilution of the protein was placed in boiling water for fifteen minutes; the precipitate which formed was removed by centrifugation. Serial titrations were then made of heated and unheated material utilizing them as antigens in complement-fixation tests with five positive serums. The results are shown in

Table 5. The procedure of heating the acid-soluble protein resulted in the precipitation of inactive material, but did not significantly alter the complement-fixing properties of the antigen.

The solubility of the active component was next tested utilizing acetone, 95 per cent ethyl alcohol, and absolute methyl alcohol as precipitants. For this purpose, four volumes of each reagent were added to one volume of a 1:100 dilution of antigen E (Witebsky). The tubes were permitted to stand at 4 C. overnight and were centrifuged the following morning. The precipitates were washed twice with homologous precipitant and brought into solution by gentle

TABLE 5

COMPLEMENT-FIXATION TESTS WITH ACID-SOLUBLE PROTEIN (ORIGINAL DILUTION, 1:1000). EFFECT OF HEATING THE ANTIGEN IN INDICATED DILUTIONS

LOT OF SERUM (DILUTED 1:10)	FURTHER DILUTION OF UNHEATED ANTIGEN						FURTHER DILUTION OF HEATED ANTIGEN					
	1:1	1:2	1:4	1:8	1:16	1:32	1:1	1:2	1:4	1:8	1:16	1:32
1	C*	AC	W	O	O	O	C	C	O	O	O	O
2	C	C	O	O	O	O	C	AC	O	O	O	O
3	C	C	C	W	O	O	C	C	C	O	O	O
4	C	C	P	O	O	O	C	AC	W	O	O	O
5	C	C	C	C	W	O	C	C	C	C	W	O

* C, complete fixation; AC, almost complete fixation; P, partial; W, weak; O, complete hemolysis. Antigen controls were included.

TABLE 6

COMPLEMENT-FIXATION IN TRICHINOSIS: PRECIPITATION OF ANTIGEN (ORIGINAL DILUTION 1:1000) BY ACETONE AND ALCOHOL

PRECIPITATING AGENT	TITER OF PRECIPITATE					TITER OF SUPERNATE				
	1:2	1:4	1:8	1:16	1:32	1:2	1:4	1:8	1:16	1:32
None	C*	C	C	AC	O					
Acetone	AC	C	C	AC	P	O	O	O	O	O
Ethyl alcohol	C	C	C	AC	O	O	O	O	O	O
Methyl alcohol	AC	C	C	AC	O	O	O	O	O	O

* C, complete fixation; AC, almost complete fixation; P, partial fixation; O, complete hemolysis. Anticomplementary controls showed complete hemolysis.

warming in saline. They were then diluted and utilized as antigens for complement-fixation tests. The supernates were diluted in a similar fashion and tested with a known positive serum in the same experiment (Table 6). Under the conditions of the experiment, it was found that the component or components responsible for the complement-fixation test in trichinosis were completely precipitable by acetone, ethyl alcohol and methyl alcohol.

Additional experiments of preliminary character indicate that the complement-fixing antigen is probably a polysaccharide with properties differing from the one described by Melcher.¹ Studies to determine the nature of this substance are in progress.

DISCUSSION

Studies of fractions derived from *Trichinella spiralis* larvae have revealed that serologic activity in the human disease is possessed by some and lacking in others. While the antigens utilized in this study have been classified as "pure," they are in reality, relatively crude extracts of heterogeneous composition. The polysaccharide, which proved to be almost inactive was, in all probability, the most highly purified substance tested. The acid-soluble protein was obtained by precipitation of the acid-insoluble material from an alkaline borate buffer extract of defatted larvae¹ and may have contained polysaccharide as well. The fact that serologic responses were elicited in the rabbit with these fractions may merely indicate species differences to immunization with larvae. In fact, Augustine and Theiler¹ pointed out that only the delayed wheal appeared in the skin of sensitive guinea pigs and rabbits.

As a result of the experience gained in the study of this epidemic, it is believed that a major component of *Trichinella spiralis* larvae responsible for intradermal, precipitin, and complement-fixation reactions is heat-stable and precipitable by alcohol and acetone. These facts may be applied to the solution of practical clinical problems in the following manner. It should now be possible to extract defatted larvae with hot alkaline saline (pH 8.0) in order to eliminate less reactive, heat-precipitable material present. The clarified extract could then be treated with sufficient acetone or alcohol to induce maximum precipitation in the cold (4 to 5 volumes). The precipitates could be washed, redissolved in saline or water and reprecipitated until a readily soluble product was obtained. The final precipitate would then be dried over ether and weighed. For purposes of serologic testing, dilutions of exact amounts of material could be standardized by intradermal means and against serums from known cases of trichinosis for skin, precipitin, and complement-fixing activity. This standardization would serve as a reference point for all future extracts and reactions. Preparation of material for clinical use would be simplified by the stability of the substances involved and by the utilization of heat for sterilization purposes.

SUMMARY

Intradermal, precipitin, and complement-fixation tests were performed on 120 individuals, twelve weeks after the onset of symptoms of trichinosis. For this purpose, an alkaline saline extract, an acid-soluble protein, and a polysaccharide were utilized. Although highly concentrated, these fractions were found to be less sensitive than the crude extracts of *Trichinella spiralis* commonly employed. The serologically active substance or substances were heat-stable and precipitable by alcohol and acetone. As a result of these studies, a practical method of standardizing reagents for serologic tests in trichinosis is formulated.

Acknowledgments. We wish to thank Dr. L. R. Melcher and Dr. E. Witebsky for furnishing the antigens used in this study; Brigadier General Joseph E. Bastion for his encouragement and interest; 1st Lt. (SnC) C. Becker and the following enlisted men for their invaluable voluntary technical assistance: A. S. Resnick, T/Sgt.; R. Kramer, T/4; O. Bower, Pfc.; C. Childs, T/4; S. Kennedy, T/5; and R. Brotherton, Pfc.

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DEMONSTRATION IN SKIN OF ACTIVE CHLORINE FROM CHLORINE-LIBERATING OINTMENTS*

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During World War II, a great amount of work was done to develop the least irritating chlorine-liberating ointment which would protect the exposed skin against liquid mustard or its vapor. In the course of this work, the question arose as to whether the liberated chlorine penetrated the skin, and if so, to what extent. This question was answered by demonstrating the presence of free chlorine in frozen sections of skin by the use of an orthotolidine reagent.

Orthotolidine has long been used to detect free chlorine in water. The reagent, as used in water tests, is a 0.1 per cent solution in 10 per cent hydrochloric acid. A yellow to orange color develops, depending on the concentration of chlorine. However, when a weak acid, such as citric or tartaric, is used to dissolve the reagent, the color produced with small amounts of chlorine is clear blue-green, and yellow or orange with large quantities of chlorine.

In the usual preparation of histologic specimens by embedding in paraffin, the tissues must be dehydrated with alcohol and chloroform. This procedure destroys or liberates any free chlorine in the tissues. Therefore, the frozen section method was chosen.

EXPERIMENTAL PROCEDURES

Reagent. The reagent was prepared by dissolving 0.1 gm. o-tolidine in a solution of 1 gm. citric acid in 20 ml. water, and increasing the volume to 100 ml. with distilled water. The pH of this solution was 1.86.

Technic. The chlorine-liberating ointment was spread evenly over the depilated area of the experimental animal's abdomen and allowed to remain for a few minutes. A section of this area was taken for biopsy, without removing the ointment, and the specimen was dipped in boiling formalin for two seconds. This small piece of fixed tissue was frozen and sectioned at a thickness of 25 microns. Sections were placed in the o-tolidine reagent for fifteen to twenty-five seconds, and mounted on a glass slide in glycerin.

Twelve adult rabbits and twelve adult guinea pigs were used. The anterior abdominal wall was depilated with strontium sulphide twenty-four hours before the ointment was applied, to avoid unnecessary irritation. Two experimental ointments‡ were used for comparison. One-half gm. of ointment A was applied to an area 10 cm. long and 5 cm. wide on the right side of the abdominal wall.

*Received for publication, August 23, 1946.

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‡The formulas of these chlorine-liberating ointments cannot be disclosed at this time for reasons of security.

Similarly ointment B was applied on the left side. The ointments were spread with the finger, each being applied with the same number of strokes and, as nearly as possible, with the same amount of pressure.

The same technic was used for the guinea pigs, except that 0.2 gm. of each ointment was applied over one-third of the surface area used in the rabbits.

Two rabbits and two guinea pigs were sacrificed at each of the following periods: thirty minutes, one, two, three, five and twenty-four hours. Three strips of skin, each 0.5×4 cm. in area, were taken from each side of the abdominal wall. These sections were then treated by the technic described above.

RESULTS

In this study, the presence of a green color was interpreted as indicating small amounts of chlorine from the ointments, while a yellow or orange color indicated large amounts of chlorine.

The results of the experiments with guinea pigs and rabbits were practically identical. In the animals killed after thirty minutes, the chlorine of ointment A appeared in the stratum corneum, the stratum granulosum and the stratum germinativum, while the chlorine of ointment B penetrated only as far as the stratum granulosum. The penetration of the latter ointment was deeper at the end of the first and second hours. At the end of the three and five hour periods, the chlorine from ointment B, as well as from ointment A, had penetrated all four layers of the epidermis. In no section, however, was it possible to demonstrate chlorine in the dermis. Both ointments had a tendency to follow the hair follicles.

At the end of twenty-four hours, all the free chlorine in ointment B had disappeared from the skin, except for a slight amount in the stratum corneum and in a few hair follicles. With ointment A, no free chlorine could be demonstrated at this time except in an occasional hair follicle.

DISCUSSION

From the data given above, it appears that the chlorine from ointment A penetrated the skin more rapidly than that from ointment B. The chlorine from the latter remained in the skin longer than did that from the former, probably owing either to the lower rate of decomposition, or to the greater initial chlorine content of the former ointment.

SUMMARY

The presence of free chlorine in the skin was demonstrated by the use of an o-tolidine-citric acid reagent on frozen sections. When ointments containing active chlorine were applied to the skin of rabbits and guinea pigs, the chlorine penetrated no deeper than the basement membrane. A comparison of two chlorine-containing ointments showed differences in rate and duration of penetration of chlorine.

A METHOD FOR THE VISUAL DEMONSTRATION OF LEWISITE IN SKIN*

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During World War II, in the course of work on the action of lewisite (dichloro-2-chlorovinyl arsine), it was found desirable to follow visually the penetration of this compound into the skin. A method was therefore devised which could demonstrate the existence of lewisite, or its oxide (2-chlorovinyl arsenic oxide), in the skin at various periods of time after application of liquid lewisite. This method was specific for lewisite or its oxide and was not affected by any other decomposition product.

EXPERIMENTAL PROCEDURES

Principle. In alkali, lewisite and its oxide decompose to liberate acetylene. This combines with cuprous salts to form cuprous acetylides, a red compound, the color of which is enhanced by piperidine. Only acetylene-forming compounds can produce this compound. Other degradation products of lewisite, such as oxides of arsenic, will not react with the reagent.

Reagents. The following stock solutions‡ were prepared:

A. Copper sulfate crystals.....	8 gm.
Water.....	100 ml.
B. Anhydrous sodium sulfite.....	20 gm.
Water.....	100 ml.
C. Sodium thiosulfate.....	50 gm.
Sodium hydroxide.....	20 gm.
Water.....	80 ml.
Piperidine.....	1 ml.

Ten ml. of solution A and 10 ml. of solution B were mixed until the precipitate which first formed, redissolved and the solution became light clear yellow. Twenty ml. of solution C was then added. The work here reported was done with this mixture of solutions. Subsequent experimentation showed that more satisfactory sections could be obtained as a result of the reduction in alkali content in the following mixture: Ten ml. of solution A and an equal volume of solution B were mixed until clear and 10 ml. of solution C was added. To this was added 20 ml. of water. Both mixtures were somewhat unstable, absorbing oxygen from the air, and turning blue after several hours.

Technic. Twenty guinea pigs, each weighing between 250 and 300 gm., were used in these experiments. Each pig was tied to a board and the hair over the abdomen was clipped with triple-0 clippers to prevent irritation from shaving.

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‡These were modifications of solutions used in work on the detection of lewisite by B. Gehauf and M. M. Falkof (personal communication to the authors).

Four areas, each 1 cm. square, were marked off on the abdominal wall, just to the left of the linea alba. In the center of each square, 0.76 mg. of liquid lewisite was placed by means of a capillary pipet. Sections of skin, each 1.5 x 0.5 cm., through the contaminated areas were removed at the following intervals after the application of lewisite: two, five, eight, ten, and thirty minutes and one, two, four, five, six, sixteen and twenty-four hours.

The strips of skin were placed on the freezing microtome and sections were cut at a thickness of 50 microns. The sections were then dipped in the reagent for three to five seconds, washed in distilled water, dehydrated in absolute alcohol, cleared in xylene, and mounted in "Permunt."

Attempts were made to counterstain some of the sections with hematoxylin. In general, this was not successful and very few good sections were so obtained.

RESULTS

In sections taken two minutes after the application of lewisite, the agent was found to have entered the epidermis. Sections taken at five and eight minutes showed further penetration into the derma and, at the end of the ten minute period, the lewisite had completely penetrated the derma. It remained undecomposed for thirty minutes and then gradually disappeared from the derma. At the end of two hours, it appeared only above the stratum lucidum, except for a small amount in the hair follicles. After twenty-four hours, only traces could be demonstrated in an occasional hair follicle.

In all sections, the lewisite was observed to penetrate the skin in a straight line, perpendicular to the point of application, with little or no lateral diffusion. It penetrated between the hair follicles as rapidly as it did through the follicles.

The presence of lewisite in the underlying muscles could not be demonstrated with the small quantities of agent used.

SUMMARY

A technic for demonstrating the presence of lewisite in skin has been developed by the use of an alkaline cuprous salt reagent. Liquid lewisite penetrates completely through the skin of guinea pigs in ten minutes, and remains for about thirty minutes. Lewisite disappears from the skin of guinea pigs after approximately twenty-four hours.

A METHOD FOR THE VISUAL DEMONSTRATION OF MUSTARD (DICHLORODIETHYL SULFIDE) IN SKIN*

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While conducting work on mustard gas (dichlorodiethyl sulfide), during World War II, it was found desirable, at one point, to follow visually the fate of mustard in the skin. This paper describes the technic used and the results of experiments which were carried out. The authors have in previous communications outlined methods for demonstration of the presence of chlorine¹ and of lewisite² in skin. This work, which is a further application of the technic of using a color-producing solution that is specific for the compound sought, demonstrates unreacted or unchanged mustard in the skin.

EXPERIMENTAL PROCEDURES

Principle. When mustard and gold chloride react, a water-insoluble, bright yellow gold chloride-mustard complex is immediately formed. If this is then treated with sodium hydroxide solution the complex is reduced, and forms an insoluble jet black compound.

Solutions. Gold chloride, acid-yellow, Merck and Company, 1 per cent in water; sodium hydroxide, reagent, 5 per cent in water.

Sensitivity of mustard detector solutions. The first experiments were made to determine the sensitivity of the test. By means of the flat end of a stainless steel rod, 150 micrograms of mustard was applied to a piece of filter paper. The spot was then wet with a few drops of one per cent gold chloride solution. A yellow spot appeared where the mustard had been applied. It was then made alkaline with 5 per cent sodium hydroxide solution. A black, water-insoluble spot appeared at the site of the application of mustard. This spot had the same size and shape as the original drop of mustard. It was noted, however, that the sodium chloroaurate formed by the reaction of the excess gold chloride with the alkali gradually decomposed and, on standing for ten to fifteen minutes, slowly formed a violet-gray deposit where the paper was wet. This decomposition was prevented by washing with slightly acidulated water within one minute after applying the alkali. Wash-water containing no acid produced almost as good results.

By means of a series of steel rods of different diameters flattened at the ends, a series of seven graded mustard applications, ranging from 150 to 15 micrograms, was made on a strip of filter paper, which was then treated with gold chloride, alkali, and washed with acidulated water. On this strip, all applications were clearly visible without magnification. It is probable that the test is sensitive to less than one microgram of mustard when viewed through the microscope.

Specificity of mustard detector solutions. Possible decomposition or reaction

*Received for publication, August 23, 1946.

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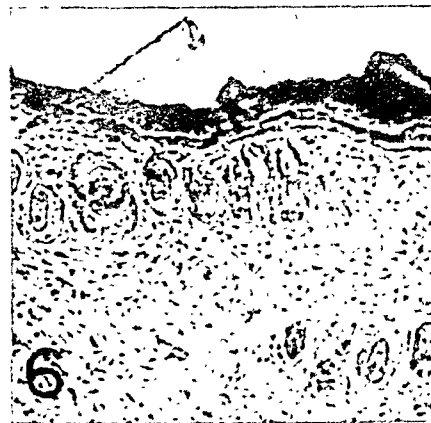
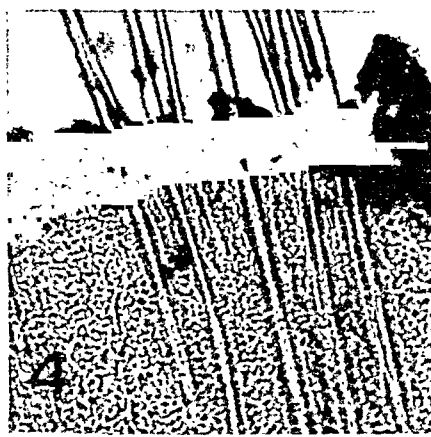
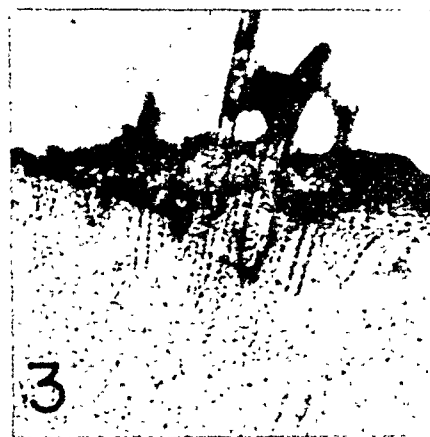
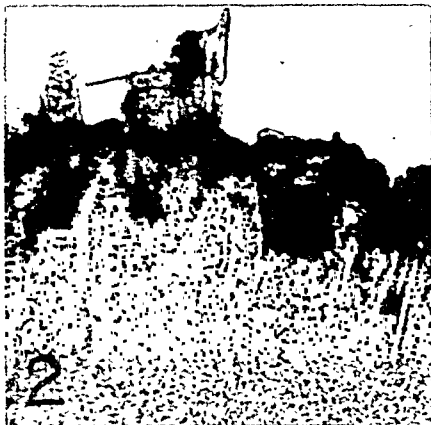
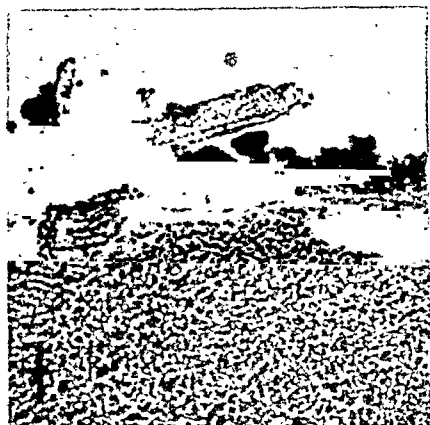


FIG. 1. Distribution of mustard in skin 30 seconds after application.
FIG. 2. Distribution of mustard in skin 1 minute after application.
FIG. 3. Distributio of mustard in 2 minutes after application.
FIG. 4. Distribution of mustar in skin 10 minutes after application.
FIG. 5. Distribution of mustard in skin 15 minutes after application.
FIG. 6. Distribution of mustard in skin 30 minutes after application

products of mustard were examined. Thiodiglycol was applied to filter paper and a few drops of gold chloride solution added. No insoluble complex was visible. On application of alkali, a diffuse black precipitate was formed over the whole wet area. This was caused by solubility in water of the thiodiglycol and its complex. If the paper was washed with water before the alkali was added, no darkening occurred. The same result was obtained when the paper was dipped in a large excess of gold chloride solution so that the thiodiglycol was washed out before the alkali was applied.

A sample of mustard-thiodiglycol sulfonium chloride was tested, both dry and in watery solution. No darkening occurred. A sample of egg albumin-mustard complex was tested as was egg albumin itself as a control. No reaction occurred with either substance.

The following protein-mustard complexes were also tested and gave no reaction: human plasma-mustard, human fibrogen-mustard, gamma globulin-mustard, cyanide process keratin-mustard, human serum albumin-mustard, and bovine albumin-mustard.

It was thus established that the test was insensitive to thiodiglycol, mustard-thiodiglycol sulfonium chloride, and to a number of mustard-protein complexes. It was highly sensitive to mustard under the same conditions.

TECHNIC

White guinea pigs, each weighing about 250 gm., were used. Each guinea pig was placed on its belly and tied securely to a board. The hair on the back was clipped and shaved. On each of four areas along the midline, a drop (1.3 mg.) of mustard was placed at intervals of approximately 1.5 cm. At various selected times, ranging from thirty seconds to two and one-half hours, a strip of skin 0.5 x 2 cm. containing the drop of mustard was excised and placed on the freezing microtome. Sections 50 to 75 microns in thickness were made and dipped into about 50 ml. of 1 per cent gold chloride solution, where they were allowed to remain for two or three minutes. From this solution each section was mounted on a slide, flooded with 5 per cent sodium hydroxide, which was allowed to remain for thirty seconds, washed thoroughly with water, dehydrated with absolute alcohol, cleared with xylol and mounted in "Permunt." The prepared sections were then examined under the microscope at a magnification of 100.

A series of sections was taken at the following intervals after application of mustard: thirty seconds, one, two, ten, fifteen, thirty minutes and one, one and one-half, two, two and one-half hours. A section was also taken of normal skin uncontaminated with mustard and similarly processed, as a control. Photomicrographs were taken of all sections within ten minutes after preparation.

RESULTS

By means of the above technic, a black coloration appeared at the site of the unchanged or uncombined mustard. In this way, its distribution could be seen in the skin sections at the various times after application (Figs. 1-12).

Within the first thirty seconds, the mustard had penetrated the epidermis and

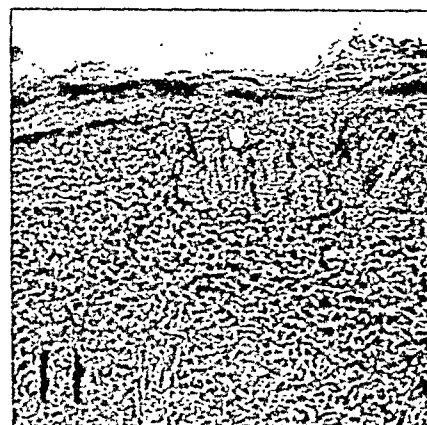
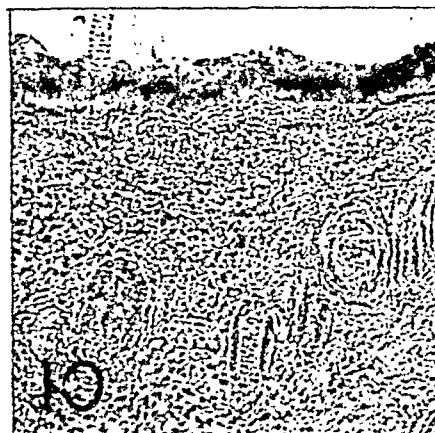
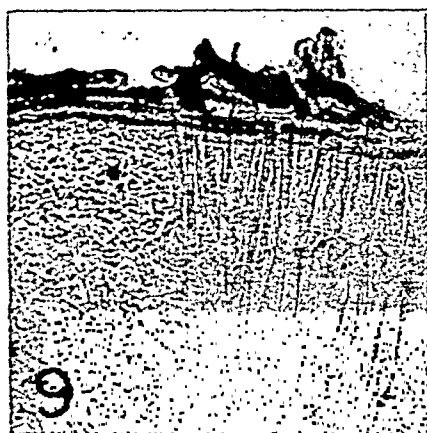
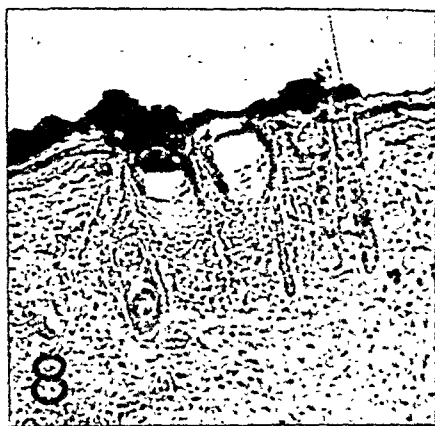


FIG. 7. Distribution of mustard in skin 45 minutes after application.
FIG. 8. Distribution of mustard in skin 1 hour after application.
FIG. 9. Distribution of mustard in skin $1\frac{1}{2}$ hours after application.
FIG. 10. Distribution of mustard in skin 2 hours after application.
FIG. 11. Distribution of mustard in skin $2\frac{1}{2}$ hours after application.
FIG. 12. Normal skin (control).

could be demonstrated in the derma. The mustard did not penetrate to any great depth in the derma and, at the end of one minute, appeared to have reached its maximum depth. After ten minutes, the free mustard had disappeared from the derma, although remaining in the epidermis. The quantity of unchanged mustard remaining in the epidermis decreased gradually until, at the end of two and one-half hours, it was gone completely.

Mustard, like lewisite,² appeared to have no special predilection for the hair follicles, penetrating as well between the follicles as through them.

DISCUSSION

The black coloration caused by the mustard reaction was permanent in character. In some cases, when the sections were not washed well, the parts of the tissue which were not contaminated with mustard turned red after standing several hours. This was probably due to the precipitation of colloidal gold from the residual traces of gold chloride which were not completely washed out. That this color was not caused by mustard was proved by the fact that normal tissue similarly processed also turned red on standing, if not washed thoroughly. The use of photomicrographs gave permanent records, uncomplicated by further color changes.

The rapid disappearance of free mustard in the derma can be ascribed to fixation or transportation while the diminution of free mustard in the epidermis is due either to evaporation or transportation into the derma and almost immediate fixation therein.

SUMMARY

The presence of free mustard in the skin was demonstrated by the use of gold chloride solution and alkali on frozen sections. Several experiments were performed to show the specificity of the method for mustard and the nonreactivity of the solutions with mustard-protein complexes and other reaction-products of mustard. The process described, although not quantitative, is simple, rapid, and capable of useful interpretation.

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MODIFICATIONS OF MUELLER'S MEDIUM FOR RAPID DIAGNOSIS OF *C. DIPHTHERIAE**

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In 1945, Mueller³ introduced a serum tellurite medium for the isolation of *Corynebacterium diphtheriae*. We had an opportunity to evaluate this medium in a community having many cases of diphtheria. In the course of time, we found it possible to modify the medium and, we believe, to render it more specific. It also became possible for us to shorten the time necessary for positive identification of the organism.

I. OPTIMUM CONCENTRATION OF TELLURITE SERUM

The standard Mueller's medium is prepared as follows: 22.5 gm. of Bacto-Mueller tellurite base is added to 500 cc. of cold distilled water in a flask. The mixture is heated to boiling over a free flame with frequent shaking of the flask, and then is permitted to simmer for three minutes until there is complete solution. The solution is sterilized at 10 lb. pressure (116 C.) for ten minutes, cooled to 50 C., and to it is added 12.5 cc. of Bacto-Mueller tellurite serum. The mixture is well shaken and then poured into sterile petri dishes. The plates are allowed to stand thirty minutes with the covers partly removed. They are then covered and set aside for twenty-four hours before use. It is important that the surface of the plates be quite dry when inoculated.

The medium was designated by the letter A, B, C, or D, depending on the amount of tellurite serum which was added, as follows: Mueller A, 6.0 cc.; Mueller B, 12.5 cc.; Mueller C, 30.0 cc.; and Mueller D, 40.0 cc.

Mueller A was abandoned because the amount of tellurite was insufficient to produce a black colony in twenty-four hours.

Mueller B contained 2.5 per cent of tellurite serum and was the standard medium. Mueller B and C were compared in 23 cultures of type *mitis*, 10 cultures of type *gravis*, 11 cultures of *C. xerosis* and 9 cultures of *C. pseudodiphthericum*. Mueller B and D were compared in 31 cultures of type *mitis*, 18 cultures of type *gravis*, 12 cultures of *C. xerose* and 14 cultures of *C. pseudodiphthericum*. Plates of these mediums were used both for the initial inoculation with the unknown throat and nose cultures and for the transplant of the organisms in pure culture. It was found that increasing the tellurite to the amounts present in C and D did not inhibit the growth of *Corynebacterium* as compared to that found on B. On the other hand, while cocci grew as rapidly on B as on D, their growth was inhibited on C in 21 cases. On both C and D, the colonies of *Corynebacterium* were distinctly blacker and more sharply outlined. It was therefore believed that the optimum concentration of tellurite serum was that present in Mueller C, since there was no inhibition of *Corynebacterium*; there was distinct

*Received for publication, June 24, 1946.

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inhibition of cocci; the colonies were distinctly blacker and it was easier to work with such colonies. Thus, the standard Mueller medium was modified to contain 30 cc. of tellurite serum instead of 12.5 cc. present in the standard medium.

II. MUELLER-DEXTROSE AND MUELLER-SUCROSE MEDIUMS

In an attempt to obtain earlier bacteriologic diagnosis of *C. diphtheriae* for the clinician, the principle of Clauberg's medium¹ was utilized. In Clauberg's medium, dextrose is added to a blood tellurite-cystine mixture with wasserblau as indicator. Clauberg thought that the addition of the dextrose would differentiate *C. diphtheriae* from *C. pseudodiphthericum* (*C. hofmannii*). We thought that it might be possible to obtain early bacteriologic diagnosis of *C. diphtheriae* by the addition of dextrose and sucrose to different plates of Mueller's medium. After many trials, Andrade's indicator was chosen as giving the best contrast to the black colonies. The optimum amount of sugar was found to be 1 per cent and of Andrade's indicator, 2 per cent. Mueller-dextrose and Mueller-sucrose mediums were prepared as follows:

Sugar solution:

1. 5 gm. of dextrose or sucrose was dissolved in 50 cc. of distilled water.

Andrade's indicator:

1. 5 gm. of acid fuchsin was dissolved in 100 cc. of distilled water.
2. 20 cc. of N/1 NaOH was added slowly with intermittent shaking.
3. The solution was allowed to stand for approximately one hour, preferably overnight.
4. The solution was sterilized and stored in the refrigerator.

Mueller-dextrose and Mueller-sucrose:

1. 22.5 gm. of Mueller tellurite base was added to 450 cc. of distilled water, and heated slowly to boiling with constant stirring.
2. The previously prepared sugar solution was now added, followed by 10 cc. of Andrade's indicator.
3. The solution was autoclaved at 10 lb. for ten minutes.
4. It was then cooled to 56 C. in a water bath.
5. Thirty cc. of Mueller tellurite serum was added and the solution was poured into sterile petri dishes.
6. The plates were allowed to stand thirty minutes with the covers partly removed. They were then covered and set aside for twenty-four hours before use.

Thirty-two hundred routine cultures from the nose and throat were studied by seeding the material from each source, each on a plate of Mueller-dextrose and of Mueller-sucrose. Positive cultures were transplanted to second plates of Mueller-dextrose and Mueller-sucrose, then to fermentation mediums* containing dextrose, sucrose and starch and to a slant of brain-heart infusion agar for virulence tests. Eighty-four cultures of *C. diphtheriae* were thus isolated.

*Fermentation Mediums. Extract. Difco Manual, 7th Edition, 1943, 170 pp.

- | | |
|---|----------|
| 1. Tryptose..... | 10 gm. |
| 2. Sodium chloride..... | 5 gm. |
| 3. Andrade's indicator (prepared as previously described) | 10 cc. |
| 4. Carbohydrates (dextrose, sucrose, soluble starch) | 10 gm. |
| 5. Distilled water..... | 1000 cc. |

A. *Morphology of colonies and organisms, and color characteristics of plates of C. diphtheriae (Figs. 1-8).*

C. diphtheriae produces, in most cases, characteristic black or dark gray colonies of distinctive morphology as outlined in Table 1. These colonies on the dextrose plate are outlined against a pink background while no discoloration occurs on the sucrose plate. In a small number of cases both initial plates are discolored pink after twenty-four to thirty-two hours, or the dextrose plate is questionably discolored pink with no change in the sucrose plate. Transplanting on second dextrose and sucrose plates gives the typical color changes in twenty-four hours on the subculture. After thirty-two hours, the color changes of the plates are found to be unreliable in view of growth of organisms other than *Corynebacterium*. An occasional case of *C. diphtheriae* (usually *minimus*³) gives no color changes on the plates. It must be pointed out that occasionally the colonies of *C. diphtheriae* resemble those of *C. xerose* and *C. pseudodiphthericum*. It is of further interest that the colonies are relatively small and less numerous on the dextrose plate and distinctly enlarged on the sucrose plate as compared with the non-sugar-containing Mueller plate. The exact explanation for this remains to be elucidated.

On gram stain* *C. diphtheriae* grown on Mueller-sucrose is gram-negative or gram-labile. Only occasionally is it gram-positive (Table 2). This is in contrast to *C. xerose* and *C. pseudodiphthericum* which are almost always gram-positive. Often the organisms of *C. diphtheriae* are gram-negative, while containing one or

*Gram Stain (Reference: TM 8-227, pp. 147-148, U. S. Army)

A. Preparation of stock solutions:

1. Crystal violet..... 13.87 gm. in 100 cc. 95 per cent ethyl alcohol
2. Safranin..... 3.41 gm. in 100 cc. 95 per cent ethyl alcohol

B. Working solutions:

1. a. Crystal violet, stock solution..... 5 cc.
- b. 95 per cent alcohol..... 5 cc.
- c. Ammonium oxalate, 1 per cent aqueous solution..... 40 cc.
2. Grams iodine:
 - a. Iodine..... 1 gm.
 - b. Potassium iodide..... 2 gm.
 - c. Distilled water..... 300 cc.
3. Decolorizer:
 - a. 95 per cent alcohol..... 50 cc.
 - b. Acetone..... 50 cc.
4. Safranin:
 - a. 10 cc. stock solution, 90 cc. distilled water.

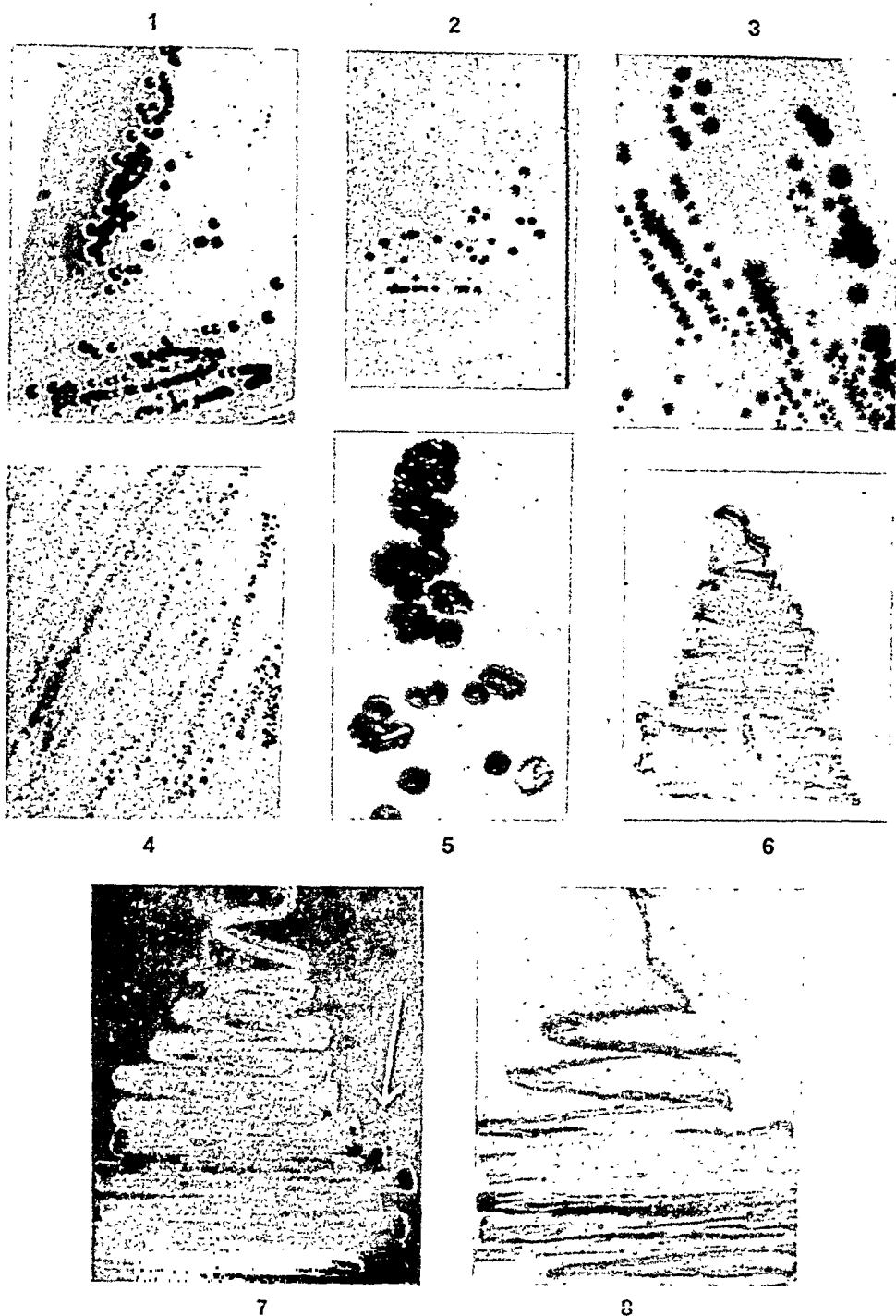
C. Procedure:

1. Crystal violet one to two minutes
2. Wash with tap water.
3. Grams iodine one minute
4. Wash with tap water.
5. Decolorize until no further traces of stain are washed out.
6. Wash with tap water.
7. Safranin one-half to one minute
8. Wash with tap water.
9. Blot dry.

TABLE 1

CRITERIA FOR DIFFERENTIATION OF *C. diphtheriae*, *mitis* AND *gravis* AND *C. pseudodiphthericum* ON MODIFIED MUELLER'S MEDIUMS

APPEARANCE OF COLONIES ON DESIGNATED MEDIUM:	<i>C. diphtheriae, mitis</i> (Figs 1, 2)	<i>C. diphtheriae, gravis</i> (Figs 3, 4, 5, 6)	<i>C. pseudodiphthericum</i>	<i>C. xerose</i>
I. Mueller's modified serum tellurite with sucrose. (Growth on dextrose plate, not characteristic.)	Smooth, convex, medium-sized. Black center and narrow semi-translucent gray periphery. Fairly abundant, moist, tend to glisten. Average diameter rarely exceeds 0.5 to 1.0 mm.	Medium to large with black convex pinhead center. Periphery three times larger than <i>mitis</i> , changing in color from center, black-gray to gray. Periphery opaque, fanned out like rays. Fairly abundant, a shade drier than <i>mitis</i> . Not glistening. Average diameter rarely exceeds 1 to 2 mm.	Medium to large, frequently rosette shaped. Striations and flat dry surface. Black, lacks luster, narrow opaque striated gray-black periphery. Not abundant, tend to spread. Average diameter rarely exceeds 2 to 3 mm. On subculture, often ferments like <i>gravis</i> and <i>mitis</i> .	Resembles <i>gravis</i> or <i>mitis</i> .
II. Color on Mueller-dextrose.	<i>C. diphtheriae (gravis and mitis)</i> slightly stunted in growth with a marked area of pink around portion of plate where streaking was made. Color demarcation from other areas sharp and uniform.		Retarded in growth, no color.	Retarded in growth but with characteristic <i>mitis</i> or <i>gravis</i> morphology, with a pink area on that portion of the streaked plate.
Color on Mueller-sucrose.	<i>Gravis</i> and <i>mitis</i> consistently increased growth with noticeable absence of color reaction on medium.		Frequently rosette shaped, with increased growth and without surrounding color.	Consistently increased growth as that of <i>gravis</i> and <i>mitis</i> and characteristic pink area around streaked portion of the plate with a sharp color demarcation.



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FIG. 1. TYPICAL COLONIES OF *C. diphtheriae mitis* ON MODIFIED MUELLER-SUCROSE MEDIUM. $\times 2$.

FIG. 2. COLONIES OF *C. diphtheriae mitis* ON MODIFIED MUELLER-DEXTROSE; INHIBITED GROWTH OF SAME STRAIN AS IN FIG. 1. $\times 2$.

FIG. 3. TYPICAL COLONY OF *C. diphtheriae gravis* ON MODIFIED MUELLER-SUCROSE. $\times 2$.

FIG. 4. COLONIES OF *C. diphtheriae gravis* ON MODIFIED MUELLER-DEXTROSE; INHIBITED GROWTH OF SAME STRAIN AS IN FIG. 3. $\times 2$.

FIG. 5. ROSETTE SHAPED CULTURE OF TYPE *gravis*, RESEMBLING *C. pseudodiphthericum*. Subculture shows typical colony and fermentation reactions of type *gravis*. $\times 2$.

FIG. 6. SAME STRAIN AS FIG. 5 SHOWING INHIBITED GROWTH ON MODIFIED MUELLER-DEXTROSE. $\times 2$.

FIG. 7. SUBCULTURE OF TYPE *gravis* ON MODIFIED MUELLER-SUCROSE AFTER 24 HOURS. Colonies are still gray with a beginning black discoloration at the edge (arrow). $\times 2$.

FIG. 8. SUBCULTURE OF SAME STRAIN AS FIG. 7, ON MODIFIED MUELLER-DEXTROSE. Note marked inhibition of growth. $\times 2$.

more gram-positive or gram-metachromatic granules. The typical clubbed, barrel-shaped and granular forms are almost always found. Only occasionally does the organism have the form of a straight long or short bacillus.

B. Time of recognition of *C. diphtheriae*

In Table 3, it is noted that 76 (90.5 per cent) cases were diagnosed as positive in from sixteen to twenty-four hours, 7 (8 per cent) in forty-eight hours, and one case remained doubtful until found positive on virulence test. Of those diagnosed in from sixteen to twenty-four hours, the majority were recognized in eighteen hours. In a more detailed study of a limited number of cases, the following results were obtained. Of 172 throat cultures taken on the same day, 14 positive cultures were obtained. Of these, two were recognized in sixteen hours, 7 (total) in nineteen hours, 9 (total) in twenty-four hours, and 14 in thirty-two hours. It is thus clear that in 90 per cent of the cases a diagnosis of *C. diphtheriae*

TABLE 2
REACTION TO GRAM STAIN OF *C. diphtheriae*, FROM MUELLER'S SUCROSE PLATE

	GRAM-NEGATIVE REACTION	GRAM-POSITIVE REACTION	GRAM REACTION DOUBTFUL	TOTAL
Number of cases.....	34	6	44	84
Percentage.....	41	7	52	100

TABLE 3
TIME REQUIRED FOR ISOLATION OF *C. diphtheriae*

	FROM 16 TO 24 HOURS	FROM 24 TO 48 HOURS	AFTER 48 HOURS FOLLOWING VIRULENCE TEST	TOTAL
Number of cases.....	76	7	1	84

can be rendered the clinician in from sixteen to twenty-four hours and in 98 per cent of the cases a diagnosis may be obtained within forty-eight hours.

C. Comparison between fermentation reactions on Mueller-dextrose and Mueller-sucrose mediums, and reactions on broth sugars of *C. diphtheriae*

This comparison is recorded in Table 4. It will be noted that the reactions on the plates proved to be far more reliable than those in the broths. This is owing to the large number of positive sucrose fermentations obtained in broth which were not consistent with virulence tests. The reason for this is at present not clear to us.

D. Cases of *C. diphtheriae* with atypical or inconclusive fermentation reactions on Mueller-dextrose and Mueller-sucrose mediums (Table 5)

In three cases the dextrose plate was insufficiently changed in color in twenty-four hours, but subcultures on sugar plates revealed the typical reactions of *C.*

diphtheriae. In five cases a faint pinkish discoloration occurred in the sucrose plate in twenty-four hours. In four of these, subcultures on sugar plates showed the typical reactions of *C. diphtheriae*. One case was not subcultured in this manner. In only one case a morphologically suspicious colony of *C. diphtheriae* gave fermentation reactions of *C. pseudodiphthericum* in the plate.

E. Control cases

Thirteen cultures of *C. pseudodiphthericum* and *C. xerose* isolated on Mueller sugar plates were compared with the fermentation reactions after inoculation on sugar broths (Table 6). Both mediums showed complete agreement as to the presence or absence of color changes. The virulence test was negative in all the examined control strains.

TABLE 4

COMPARISON OF CHANGE OF COLOR PRODUCED IN SUGAR PLATES AND IN SUGAR BROTH

	POSITIVE IN PLATES AND BROTH*	POSITIVE IN PLATES NEGATIVE IN BROTH†	POSITIVE IN BROTH NEGATIVE OR DOUBTFUL IN PLATES	ATYPICAL COLOR CHANGES IN PLATES AND IN BROTH‡
Number of cases.....	58	23	2	1

* Positive indicates fermentation of dextrose; no change of sucrose.

† Negative indicates diphtheroids or *C. xerose*.

‡ *C. diphtheriae* was confirmed only by virulence test.

III. A SUGGESTED BACTERIOLOGIC PROCEDURE FOR THE DIAGNOSIS OF
C. diphtheriae

The initial swabs from the nose and throat are plated on Mueller-dextrose and Mueller-sucrose mediums. Up to six swabs can be streaked on a sugar plate. The plates are read in sixteen hours, and again in twenty, twenty-four and forty-eight hours.

If black colonies, of any shape, the size of a pinhead or larger, appear within twenty-four hours on both plates with a pink discoloration of the dextrose plate, and with no discoloration of the sucrose plate, a diagnosis of *C. diphtheriae* is made.

If colonies with a faintly pink discoloration of the dextrose plate, or if colonies having a pink or faintly pink discoloration of both plates, appear within twenty-four hours, one of the colonies from the sucrose plate is transplanted to another pair of Mueller sugar plates. If both plates are again discolored pink, a negative diagnosis is rendered. If faintly pink discolorations are again evident on one or both plates in forty-eight hours, a diagnosis of doubtful is rendered to the clinician, and the procedure outlined below is used for further identification. A pink coloration on either plate after thirty-two hours has no significance and the plates are not considered thereafter from the standpoint of color. The sucrose plate is used for further study.

TABLE 5
CASES IN WHICH REACTION ON SUGAR PLATES AFTER 24 HOURS WAS ATYPICAL.

COLOR CHANGE IN		COLOR CHANGE IN SUGAR BROTH		CONCLUSION FROM SUGAR PLATES	CONCLUSION FROM SUGAR BROTH	RESULT OF VIRULENCE TEST
Dextrose Plate	Sucrose Plate	Dextrose	Sucrose			
—	—	+	+	Direct culture: <i>C. pseudodiphthericum</i> Subculture: <i>C. diphtheriae</i>	<i>C. xerose</i>	++++
±	—	+	—	Direct culture: doubtful Subculture: <i>C. diphtheriae</i>	<i>C. diphtheriae</i>	+++
±	—	+	—	Direct culture: doubtful Subculture: <i>C. diphtheriae</i>	<i>C. diphtheriae</i>	+++
±	—	±	—	Direct culture: doubtful Subculture: <i>C. diphtheriae</i>	Doubtful	++++
±	—	+	—	Plates doubtful after 48 hours	<i>C. diphtheriae</i>	+++
+	±	+	—	Direct culture: doubtful Subculture: <i>C. diphtheriae</i>	<i>C. diphtheriae</i> . From subculture: dextrose and sucrose, positive (<i>C. xerose</i> ?)	+++
+	±	—	—	Direct culture: doubtful Subculture: <i>C. diphtheriae</i>	From direct culture: <i>C. pseudodiphthericum</i> From subculture: <i>C. diphtheriae</i>	++
+	±	+	—	Direct culture: doubtful Subculture: <i>C. diphtheriae</i>	<i>C. diphtheriae</i>	++
+	±	—	+	Doubtful color change on sucrose plate after 48 hours (virulence test indicated)	From direct culture: doubtful From subculture: <i>C. diphtheriae</i>	++
+	±	+	—	Direct culture: doubtful Subculture: <i>C. diphtheriae</i>	<i>C. diphtheriae</i>	++++

If a black or grayish black colony of any shape or size appears only after forty-eight hours on the sucrose plate, a smear and gram stain is made of the colony. If gram-positive, labile, or gram-negative rods with the morphology of *Corynebacterium* are found, further identification as outlined below is necessary. If gram-positive or labile rods not morphologically consistent with *C. diphtheriae* are found, then the clinician is consulted as to the clinical possibility of diphtheria. If the clinical picture is suspicious, further identification as outlined below is necessary.

If no black colonies are noted at the end of forty-eight hours a negative diagnosis is rendered. If black colonies are noted, a transplant is made on Mueller-sucrose medium for purification, and a subculture is then made on a brain-heart infusion (BHI) slant, and in starch broth. The BHI is used for the virulence

TABLE 6
CASES OF *C. pseudodiphthericum* AND *C. xerose* ISOLATED ON MUELLER'S SUGAR PLATE
AND COMPARED WITH THE REACTION IN SUGAR BROTH

COLOR CHANGE IN		COLOR CHANGE IN SUGAR BROTH		CONCLUSION FROM SUGAR PLATES	CONCLUSION FROM SUGAR BROTH	RESULT OF VIRULENCE TEST
Dex- trose plate	Sucrose plate	Dex- trose	Sucrose			
-	-	-	-	<i>C. pseudodiphthericum</i>	<i>C. pseudodiphthericum</i>	-
+	+	+	+	<i>C. xerose</i>	<i>C. xerose</i>	-
+	+	+	+	<i>C. xerose</i>	<i>C. xerose</i>	-
-	-	-	-	<i>C. pseudodiphthericum</i>	<i>C. pseudodiphthericum</i>	-
-	-	+	+	<i>C. pseudodiphthericum</i>	<i>C. xerose</i>	-
+	+	+	+	<i>C. xerose</i>	<i>C. xerose</i>	-
-	-	+	+	<i>C. pseudodiphthericum</i>	<i>C. xerose</i>	-
-	-	+	+	<i>C. pseudodiphthericum</i>	<i>C. xerose</i>	-
+	+	+	+	<i>C. xerose</i>	<i>C. xerose</i>	-
-	-	-	-	<i>C. pseudodiphthericum</i>	<i>C. pseudodiphthericum</i>	-
+	+	+	-	<i>C. xerose</i>	<i>C. diphtheriae</i>	-
-	-	+	+	<i>C. xerose</i>	<i>C. diphtheriae</i>	-
+	+	+	+	<i>C. xerose</i>	<i>C. xerose</i>	-

test. If the virulence test is positive, this is correlated with the reaction on starch. If the reaction on starch is acid, a diagnosis of *C. diphtheriae gravis* is made. If the reaction is not acid, a diagnosis of *C. diphtheriae mitis*, *intermedius* or *minimus* is made, depending on previous morphology of the colonies on Mueller-sucrose medium.

COMMENT

This procedure for the more rapid bacteriologic identification of *C. diphtheriae*, in our opinion, has the following advantages:

1. The use of Mueller-dextrose medium makes possible better visualization of the black colonies, clearly seen against the pink background.
2. The use of Mueller-sucrose produces larger colonies.

3. A positive bacteriologic diagnosis of *C. diphtheriae* is possible in from sixteen to twenty-four hours in about 90 per cent of the cases.

In our opinion, it is not of great value to the clinician to know the type of *C. diphtheriae* present. A simple diagnosis of *C. diphtheriae* in from sixteen to forty-eight hours is the only clinically valuable laboratory finding at this time. A more complete bacteriologic report may follow later, giving the type of organism and its virulence.

SUMMARY

A study was made of Mueller's medium using 5000 routine throat cultures. On the basis of the initial study, Mueller's medium was modified so as to contain 6 per cent, rather than 2.5 per cent, of tellurite serum.

Mueller-dextrose and Mueller-sucrose plates were then devised. With the aid of these plates, it was possible to make the bacteriologic diagnosis of *Corynebacterium diphtheriae* in 98 per cent of cases within forty-eight hours and in 90 per cent of cases in from sixteen to twenty-four hours. The fermentation reactions on Mueller plates were found to be more accurate than those obtained in broth sugars.

A procedure for the rapid bacteriologic diagnosis of *C. diphtheriae* is suggested.

Acknowledgment. Thanks are due to Miss Nancy Pohl Procter who did most of the technical work connected with this investigation.

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EFFECT OF SODIUM CHLORIDE BALANCE ON AVIDITY OF *m* AND *n* AGGLUTININS*

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In a previous paper,² the effect of the sodium chloride balance on the avidity of the isohemagglutinins *a* and *b* was discussed. This investigation concerns the effect of sodium chloride on the agglutinins *m* and *n*.

The agglutinins were produced in the usual manner,¹ using human cells OM and ON as the antigens for rabbit immunization. The rabbits were bled and the serums collected separately. Each serum was inactivated in the water bath at 56 C. for twenty minutes and diluted 1:20 with 0.85 per cent saline for absorption, and for the experiment presented in this paper. It was found that the anti-N serum required two absorptions and the anti-M one, the *m* agglutinin being produced more readily in this instance. Group OM and ON human cells were used for the absorption of the species agglutinins and all serums were neutralized by the use of group specific substances A and B, which were kindly supplied by Eli Lilly and Company for research purposes.

Tests were then set up to show the effect of the sodium chloride balance on the titer and avidity of these agglutinins. Equal amounts of a 5 per cent suspension of homologous red cells and test serum were mixed on a Boerner well slide for the avidity test. A 2 per cent suspension of homologous red cells was used in the serial titrations. Individual serums were tested, after varying amounts of solid sodium chloride had been added to the liquid serum.

In this series, the *m* agglutinin was usually strengthened by the addition of sodium chloride, but at certain levels, a marked deleterious effect was noted, even with certain low concentrations of sodium chloride. In searching for an explanation of this phenomenon, it was found that by pooling the individual serums, the results obtained were similar to those displayed by serum containing the *n* agglutinin (Table 2). The *n* agglutinin seemed radically improved and remained so, leveling off like the pooled anti-N serums reported in Table 2. There was a slight variation in the titer of the *m* agglutinin, while the *n* agglutinin titer remained constant in this individual testing group. The reason for the inhibiting effect of certain concentrations of sodium chloride on some individual *m* agglutinins is not known.

The serums were then pooled and 1 cc. dried in a small vial *in vacuo*. Individual vials of serum were restored to original volume by the addition of saline solutions, ranging from 0.1 per cent to 2.0 per cent. They were then tested for avidity and by serial titration with the results shown in Tables 1 and 2.

One or two variations occurred in the results as shown in Tables 1 and 2, when the serums were pooled. The sodium chloride in all concentrations tested, no

*Received for publication, August 16, 1946.

longer showed a deleterious effect on the *m* agglutinin and the serum seemed as much improved as the anti-*N* serum. The *m* agglutinin titer remained comparatively stable. All reactions were irreversible.

DISCUSSION

During this investigation, several other points were noted which were of interest. It is known that the agglutinins *m* and *n* are unrelated to *a* and *b*.⁴ Their reactivity on the slide is different. In the *a* and *b* agglutinin reaction, a

TABLE 1
EFFECT OF CONCENTRATION OF SODIUM CHLORIDE ON AGGLUTINATION IN POOLED
ANTI-M SERUM

	TIME REQUIRED FOR VISIBLE AGGLUTINATION	TIME REQUIRED FOR COMPLETE AGGLUTINATION		TITER
	<i>Seconds</i>	<i>Minutes</i>	<i>Seconds</i>	
Water (H ₂ O)	25	5		
Per cent NaCl				
0.1	30	5		1:32
0.2	30	5		1:32
0.3	30	5		1:32
0.4	30	4	30	1:32
0.5	30	4	30	1:32
0.6	25	2	30	1:32
0.7	20	2	30	1:32
0.8	20	2	30	1:32
0.9	25	2	15	1:32
1.0	25	2	0	1:32
1.1	25	2	0	1:32
1.2	25	2	0	1:32
1.3	30	2	0	1:32
1.4	30	2	15	1:32
1.5	30	2	15	1:32
1.6	30	2	30	1:16
1.7	30	2	15	1:32
1.8	25	2	0	1:32
1.9	25	2	0	1:16
2.0	25	2	15	1:16

quick circular motion (rotator at 90 r.p.m.), or a tilting of the slide back and forth to an angle, rapidly brought a complete agglutination. In the *m* and *n* reaction, the slowest and slightest hand motion, holding the slide in both hands, even allowing it to remain idle a few seconds, completed the reaction in the shortest possible time. The *m* and *n* reactions were not like the swift, aggressive, hard-to-break clumps of the *a* and *b* reactions.

It was noted that the more concentrated the cell suspension, the quicker and more complete the reaction. In fact, dipping an applicator stick directly into the clot, or into straight cells, and then mixing with one drop of serum, thus

obtaining quite a heavy suspension of cells, seemed to give better results than when using a standard 5 per cent suspension. This was found in the preliminary testing of cells to be used in this investigation. Further work is being carried out to confirm this observation.

Despite the slight variation of titer in the individual serial titrations of the anti-M rabbit serum, titer and avidity do not go hand in hand. It is the avidity of the particular serum used which should be the criterion of its potency and safety rather than the titer, as it has been demonstrated that while the titer remains stable, the combining strength of the antibody for the antigen has been

TABLE 2
EFFECT OF CONCENTRATION OF SODIUM CHLORIDE ON AGGLUTINATION IN POOLED
ANTI-N SERUM

	TIME REQUIRED FOR VISIBLE AGGLUTINATION	TIME REQUIRED FOR COMPLETE AGGLUTINATION		TITER
	<i>Seconds</i>	<i>Minutes</i>	<i>Seconds</i>	
Water (H ₂ O)	15	1	25	1:32
<i>Per cent NaCl</i>				
0.1	15	1	15	1:32
0.2	12	1	10	1:32
0.3	10	1	10	1:32
0.4	10	1	0	1:32
0.5	10	1	0	1:32
0.6	10		50	1:32
0.7	8		50	1:32
0.8	8		50	1:32
0.9	7		45	1:32
1.0	7		45	1:32
1.1	7		40	1:32
1.2	6		40	1:32
1.3	6		37	1:32
1.4	6		37	1:32
1.5	6		35	1:32
1.6	6		35	1:32
1.7	5		35	1:32
1.8	5		35	1:32
1.9	5		35	1:32
2.0	5		35	1:32

radically improved. The greater the combining strength of the agglutinin, the greater the possibility of picking up weak reactors and the subgroup N₂.

CONCLUSIONS

The avidity of the *m* and *n* agglutinins is radically improved by the alteration of the sodium chloride balance. A final sodium chloride concentration in pooled anti-N serum of about 1.8 per cent, and in pooled anti-M serum of about 1.15 per cent seemed optimal in this series. The agglutinin titers of anti-M and anti-N serums do not reveal the actual potency of the serums.

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EDITORIAL

THE COLLEGE OF AMERICAN PATHOLOGISTS

In a truly democratic spirit and amid manifest enthusiasm, some 140 American pathologists, many of them representing local, state or regional societies, convened in Chicago on December 12 and 13 last, to organize The College of American Pathologists. The primary purpose of this organization is to elevate the standards of practice of pathology, emphasizing those educational aspects which have not been covered by other existing national organizations of pathologists.

The need for such an organization has been apparent for some years, and with its creation, a new phase in growth has begun in the field of Pathology. While it is satisfying to realize that this body has the sanction of the overwhelming number of pathologists, it may be well to point out that the creation of this body was made possible chiefly through the encouragement and initial endorsement of the American Society of Clinical Pathologists.

In the formation and direction of this new movement, there looms foremost the spirit and guiding genius of Dr. Frank W. Hartman of Detroit, an educator in American Medicine who has already done much for the advancement of pathology. Dr. Hartman was one of the founders and a past president of the American Society of Clinical Pathologists and one of the founders, and for twelve years secretary, of the American Board of Pathology. It is fitting, therefore, that Dr. Hartman should have been acclaimed as the first president of this College. The other officers and the members of the Board of Governors have also merited confidence chiefly by their scientific accomplishment but also by their demonstration, in the past, of devotion and zeal for those high principles which attract so many men of worthy purpose to the study of pathology. It is needless to mention that any gain which may accrue to the individual pathologist, as a result of the establishment of the College, must be regarded by him only as an incidental consequence to his fulfillment of a larger purpose, viz., of his responsibility to his profession, which, in the last analysis, can only be measured in terms of service to the patient.

We extend our sincerest good wishes for the success of The College of American Pathologists and we salute our esteemed colleagues who have been entrusted with its guidance.

S. E. G.

BOOK REVIEWS

Medical Jurisprudence and Toxicology. Ed. 8. By JOHN GLAISTER, J. P., M. D., Barrister-at-law, Regius Professor of Forensic Medicine, University of Glasgow. 691 pp., 222 illus., 89 in color. \$8.00. Baltimore: The Williams & Wilkins Company, 1945.

This work of the Glaisters, father and son, now in its eighth edition, has become a medicolegal institution. From the viewpoint of the American reader, it is an exceedingly useful textbook and reference handbook. About two-thirds of the book is taken up with discussions of identification of human remains, hairs, guns and bullets, and blood stains, as well as consideration of insanity and sex offenses, death from violence, shock, burns and other matters which fall into the field of forensic medicine. The latter third of the book is devoted to the subject of poisons and poisoning. Of particular interest is the British use of chemical tests of blood in determining the extent of alcoholic intoxication, a procedure which we might very profitably adopt in our country. In recent editions the illustrations have been progressively improved. This edition contains 222 plates, 89 of which are in color. The use of color plates represents a distinct advance in publishing and both the author and the printer are to be congratulated on the faithfulness of color rendition.

The sections of the book which deal with legal procedure in England and Scotland will be only of academic interest to the American reader. The remainder of the volume, however, offers a most valuable reference work for pathologists, physicians, medical examiners and others who are confronted with medicolegal problems.

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C. W. MUEHLBERGER

The Control of Venereal Disease. By R. A. VONDERLEHR, M.D., Director, District Number 6, U. S. Public Health Service and J. R. HELLER, JR., M.D., Chief, Venereal Disease Division, U. S. Public Health Service. 246 pp. \$2.75. New York: Reynal and Hitchcock, Inc., 1946.

Dr. Vonderlehr was director of the Venereal Disease Division of the U. S. Public Health Service from 1935 to 1943, and Dr. Heller has served in that capacity since that date. The authors are, therefore, unusually well qualified to present this major health problem. This book should be considered as a sequel to *Shadow on the Land* by Surgeon General Thomas Parran, USPHS, and *Plain Words about Venereal Disease* by Parran and Vonderlehr, with projection of war-time effort into a peace-time program. It describes the dramatic discoveries which have revolutionized the medical aspects of control. It emphasizes the need, as well as the available technics for case-finding and prompt treatment, so essential to continued progress in this field. It is written primarily to inform the public of the progress which has been made in the nation-wide fight against syphilis and gonorrhea. It will prove, however, a valuable reference for physicians, health officers, nurses and social workers interested in this field. Finally, it emphasizes the need for a continually more aggressive contact program, instead of permitting a possible post-war slow-down of this control work.

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NOTICE

The Executive Committee of the American Society of Clinical Pathologists has approved the adoption of a distinctive cover design for the Journal and the Technical Bulletin beginning with the present (January 1947) issue. The new cover was designed by Miss Louise Horne, Medical Illustrator, Wayne County General Hospital, Eloise, Michigan.

In order to eliminate the confusion which has heretofore resulted from different numbering of volumes of (1) the Journal, (2) the Technical Section and (3) the Technical Bulletin, the Editorial Board has also adopted the following changes effective with the present issue:

The title page of the Technical Section will no longer bear a volume number and the pagination of this Section will be continuous with that of the rest of the Journal.

The Technical Bulletin (official name of the separate printing of the Technical Section) will be numbered Volume 8-17 and will have the same pagination as the Technical Section.

Beginning with the 1948 issues, the Journal and the Technical Bulletin will both have the same volume number.

TECHNICAL SECTION

ASPIRATION OF STERNAL BONE MARROW

TECHNIC FOR OBTAINING VOLUMETRIC READINGS, SMEARS, IMPRINTS AND HISTOPATHOLOGIC SECTIONS*

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It is the purpose of this communication to present a simple method for obtaining smears, imprints, volumetric readings and histologic sections from a single aspirated specimen from the sternal bone marrow of patients. This technic was developed as a modification of Schleicher's method^{4,8} for obtaining "gross bone marrow units."

TECHNIC

The usual aspiration technic is followed as described in detail by Limarzi² and Schleicher.⁴ For completeness, the step by step procedure will be described with the modifications which we have found to be of great practical value.

The midline of the sternal body opposite the second interspace is the usual site of puncture. The skin in this area is shaved, cleaned and prepared with anti-septic solution. The skin, subcutaneous tissue and periosteum are infiltrated with a local anesthetic. The injected area is gently massaged until there is no elevation of the tissues. The thickness of the skin and subcutaneous tissue over the site selected for puncture is marked on the needle used for injecting the local anesthetic, with the needle at right angles to the surface of the sternum. The aspiration needle† has a guard with a threaded shank which moves upward 1 mm. for each complete turn. The puncture needle is exposed for the same length as marked off on the infiltration needle; then the guard is moved upward 2 to 3 mm. This will usually be sufficient to cause penetration of the skin, subcutaneous tissue, and the outer table of cortex of the sternum. The aspiration needle is forced into the sternum with steady pressure and slight rotation with the needle at right angles to the surface at all times. A distinct "give sensation" is felt as the needle enters the medullary cavity. After the needle is firmly embedded in the medullary cavity, which may require an additional $\frac{1}{2}$ to $\frac{3}{4}$ of a turn of the needle head, the stylet is withdrawn and a 10 cc. glass syringe is attached to the needle head. The plunger is slowly withdrawn until the first drop of marrow

* Received for publication, September 12, 1946.

† Manufactured by V. Mueller and Company, Chicago, Illinois.

fluid is visualized in the capillary end of the syringe. The syringe is immediately detached, the stylet replaced, and the first drop is expressed onto a clean slide, taking care that the sterile syringe does not touch the slide. Several smears are thus made and quickly air-dried. These smears do not come into contact with anticoagulant; hence they are of greatest value for studying cytologic detail. The same 10 cc. syringe is reattached to the needle head after removing the stylet, and the plunger is rapidly withdrawn to the 6 cc. mark and held there until 1.5 cc. of marrow has been obtained. At this time, the patient usually experiences a "suction pain" which, however, is momentary and not severe. This specimen is immediately placed in a paraffin-lined vial containing a minute amount of heparin* and gently rotated several times to mix the marrow material with the heparin. The aspiration needle is removed and a tight, sterile dressing

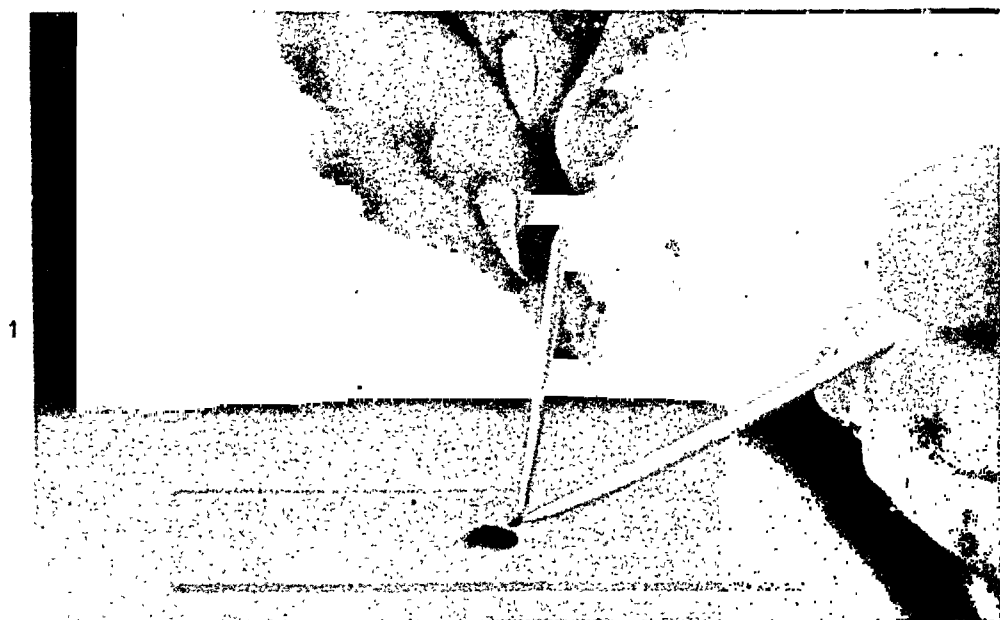


FIG. 1. TRANSFER OF GROSS MARROW PARTICLES TO PREPARED AREA ON SLIDE

is applied. The bone marrow specimen is placed in a Wintrobe hematocrit tube with the aid of a capillary pipet and centrifuged at 2,000 r.p.m. for five minutes. The marrow specimen is thus separated into four layers (fat, plasma, nucleated cells and erythrocytes) and the volume of each is recorded in "volume per cent."¹⁴ A small amount of heparinized marrow (0.5 cc.) remains in the paraffin vial.

During the centrifugation of the marrow specimen, a clean slide is prepared for collection of gross marrow particles from the remaining material. Particles are also collected from among those adhering to the barrel of the syringe, the wall of the paraffin vial and the inner surface of the capillary pipet. To prepare the slide, a small amount of powdered topical thrombin†¹⁵ is placed on the slide,

* Powdered heparin, obtained from Hynson, Westcott, & Dunning, Baltimore, Md.

† Thrombin, Topical, obtained from Parke, Davis, & Company, Detroit, Michigan.

mixed into solution with a drop of water and allowed to dry. The gross marrow particles are carefully picked up and transferred to this thrombin-coated area of the slide with the aid of the pointed ends of split wood applicators (Fig. 1). Several rows of imprints are made on clean slides from one or two of the particles.⁵ After the marrow particles have been collected and placed on the prepared

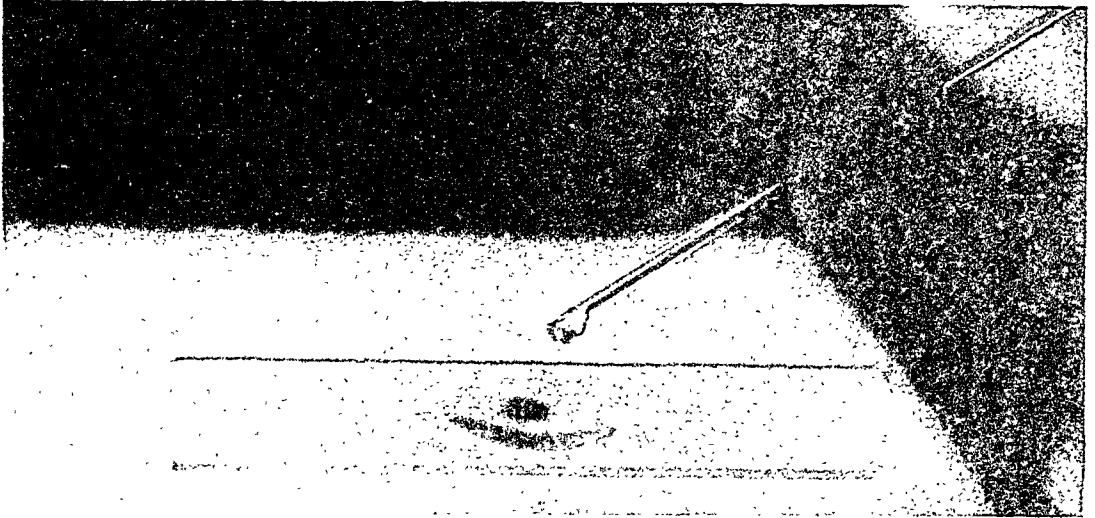


FIG. 2. ADDITION OF HEPARINIZED PLASMA TO PARTICLE CONGREGATE

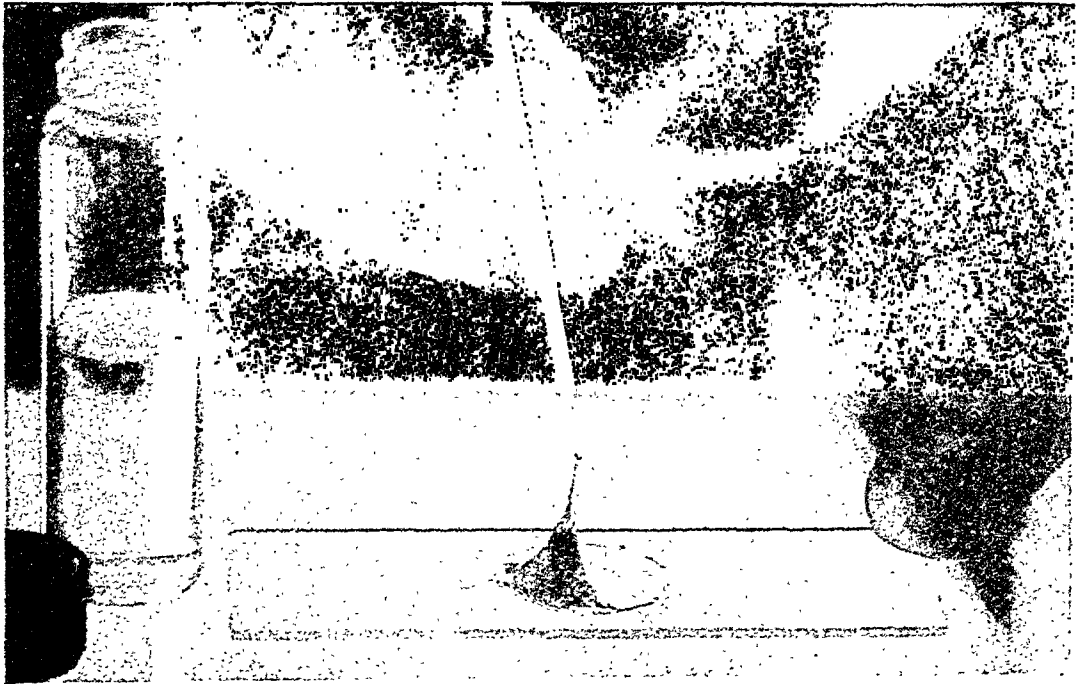


FIG. 3. PLASMA CLOT CONTAINING MASS OF MARROW PARTICLES

area of the slide, they are gently manipulated until they are in contact with each other. To this particle conglomerate are added three or four drops of the heparinized plasma obtained from the centrifuged specimen (Fig. 2). The presence of the thrombin causes clotting of the heparinized plasma. The plasma clot, which includes the compact mass of marrow particles, is now a firm mass which can be

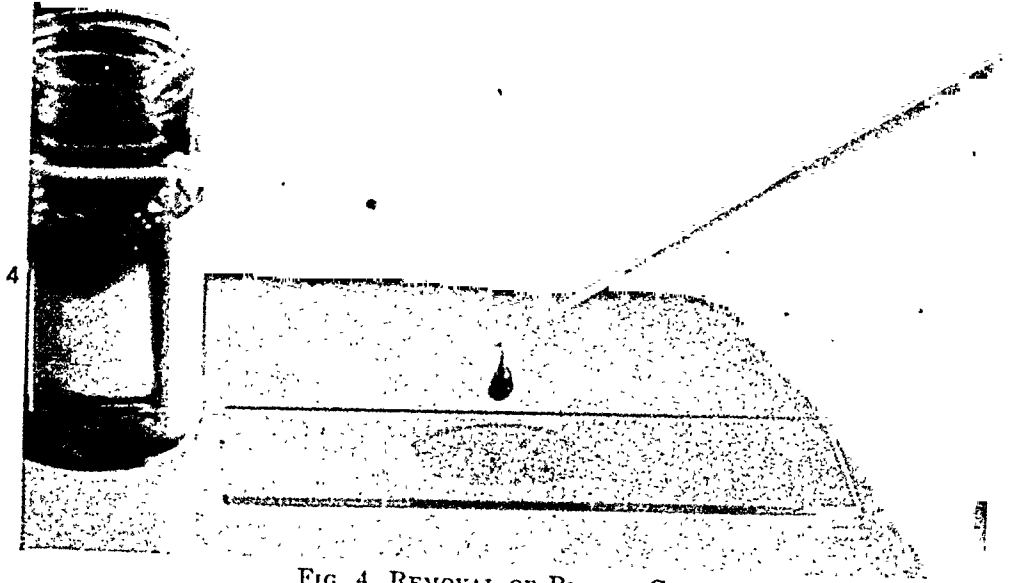


FIG. 4. REMOVAL OF PLASMA CLOT

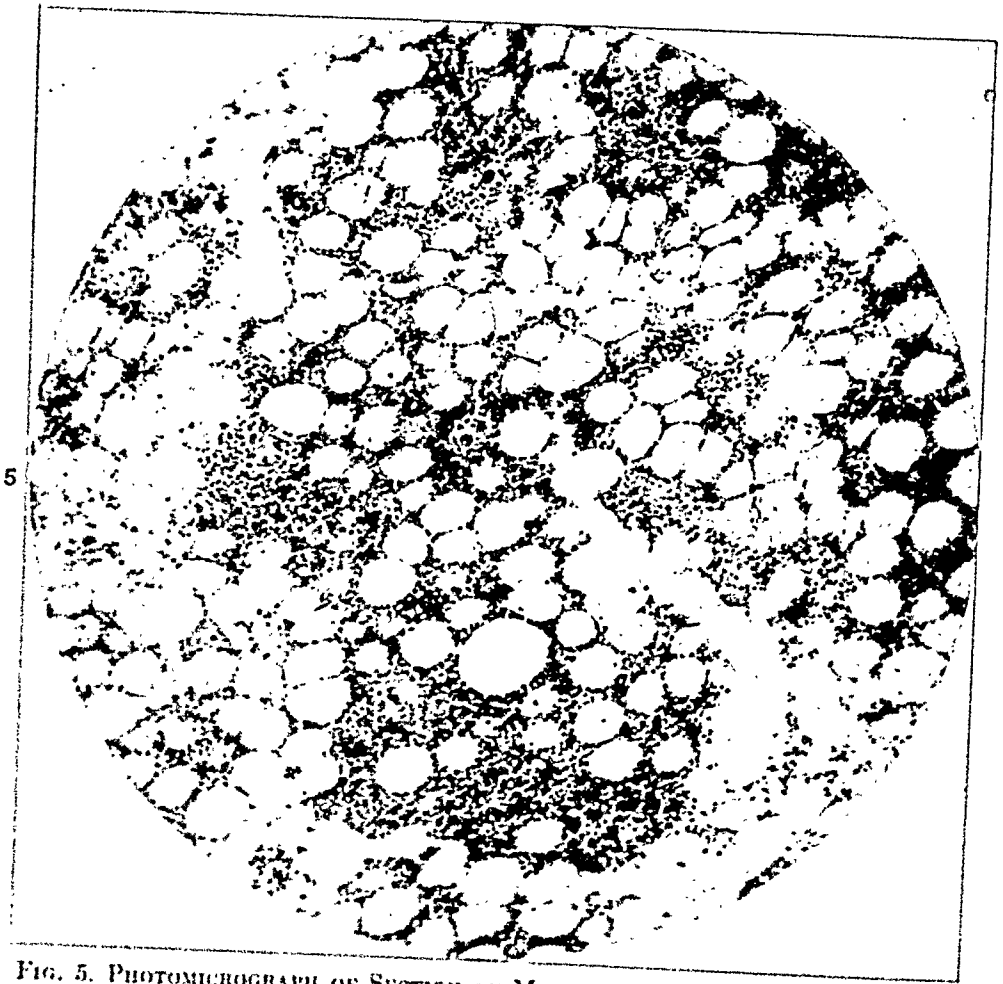


FIG. 5. PHOTOMICROGRAPH OF SECTION OF MARROW PARTICLE CONGREGATE. X75.

easily lifted from the slide and dropped into a vial containing the fixing solution⁴ (Figs. 3 and 4).

A second set of smears is made from the nucleated cell layer of the hematocrit.² An equal amount of the plasma and nucleated cells are aspirated and transferred to a paraffin-coated watch glass where they are thoroughly mixed. The smears are made from this mixture. These smears of bone marrow concentrate are used for differential counts. We have used Wright's stain⁷ or May-Grünwald-Giemsa stain⁸ with equal success. The mass of marrow particles is used for preparing histologic sections (Fig. 5).

DISCUSSION

A disadvantage of the aspiration method for obtaining bone marrow is its failure to allow for histopathologic study of bone marrow structure. Amprino and Penati,¹ in 1935, devised a method whereby the aspirated marrow material was allowed to clot in the aspirating syringe. The coagulum was then fixed, embedded in celloidin and sectioned. This method provided for histopathologic study of the bone marrow structure. Mertens,³ in 1945, using the same technic, stated that a second aspiration was necessary if smears were desired. This is a disadvantage because repeated aspirations lead to further contamination of the marrow specimen with blood⁹ and hence to inaccuracies in the differential count and volumetric pattern. Schleicher^{4, 6} and Tucker¹¹ collect the individual gross marrow "units" and prepare each particle separately for sectioning.

Using our technic as described, only 1.5 cc. of marrow material is aspirated so that dilution with sinusoidal blood is kept at a minimum. The single specimen provides adequate material for volumetric readings, smears, and imprints for cytologic study and differential counts, in addition to marrow particles from which paraffin sections can be made for observation of the bone marrow topography. The single conglomerate mass of marrow particles in the plasma clot is easily handled during the various stages of histologic preparation.

SUMMARY

A simple modification of Schleicher's technic of sternal bone marrow aspiration is described whereby material for smears, imprints, volumetric readings and histologic sections may be obtained from a single aspiration.

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SIMPLE METHOD OF PREPARING ANTI-Rh SERUM IN NORMAL MALE DONORS*

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The original method^{6, 7} of preparing anti-rhesus serum in guinea pigs has the following disadvantages: (1) the yield of serum from each immunized animal is small, and rhesus monkeys must be available to provide blood for the injections; (2) the serum cannot be used for testing newborn infants, because such blood is agglutinated by anti-rhesus immune serum, regardless of its Rh type;⁴ (3) the reactions are very delicate and, thus, satisfactory results are obtained only by highly trained technicians. The situation was much improved when human anti-Rh serum became available because this gives sharper reactions than experimental antiserum and, therefore, is more satisfactory for routine testing.

At first, human antiserum was obtained only from patients who had had transfusion reactions,¹³ or from mothers of erythroblastotic infants.⁸ As the need for serum increased, this source of supply proved inadequate, because only a small proportion of such patients possessed serum of sufficiently high titer and avidity to be useful in testing. In connection with experiments with the biological test for Rh sensitization, it was observed,¹⁶ in 1944, that two weeks following the injection of the test dose of Rh-positive blood, there was a rise in the titer of the Rh antibodies. It occurred to us that the injection of small amounts of Rh-positive blood into individuals known to be sensitized to the Rh factor might serve as a method of producing anti-Rh testing serum in human beings. Although the method was taken up by other workers^{1, 3a, 5} with considerable success, it proved uncertain because of an insufficient number of subjects who were suitable for injection. Considering the large demand for Rh antiserum, the only practicable solution seemed to lie in its production in normal, male, professional blood donors. The purpose of this communication is to describe such a procedure with which we have had success.

THEORY AND METHOD

Previous observations had shown that injections of relatively minute amounts of solutions of A and B group substances into individuals lacking the corresponding agglutinogens stimulated a pronounced rise in the alpha and beta agglutinin titers in such individuals. Among the materials successfully used were: pooled human plasma,¹⁵ in 40 to 50 cc. amounts intravenously; autoclaved human saliva,¹⁴ in quantities of 0.2 cc. intramuscularly; or minute amounts of purified A and B group substances of animal origin,¹⁷ either intramuscularly or intravenously. Yet, intravenous or intramuscular injections of relatively large amounts of Rh-positive blood into normal Rh-negative individuals failed to elicit the formation of anti-Rh agglutinins. It then became apparent that the reason for the

* Received for publication, August 15, 1946.

contrast in results following immunizing injections against A and B, in comparison with Rh, was that normal individuals have a background of sensitivity for A and B, as evidenced by the presence of natural alpha and beta agglutinins in their serum, but no background of sensitivity for Rh. The provocative effect of injections of small amounts of A or B group substance was, therefore, in the nature of an anamnestic reaction. It occurred to us that if we could build up a background of Rh sensitivity in male professional donors, a small injection of Rh-positive blood might then stimulate the appearance of potent anti-Rh agglutinins in their serums. The validity of this principle was indicated by some previous observations on intragroup hemolytic transfusion reactions.^{9, 12}

The following method of immunization was decided on for the preparation of anti-Rh agglutinating serum. Group O, Rh-positive blood was mixed with an equal volume of sterile citrate solution. The blood used in all our experiments belonged to subtype Rh₂Rh₂. Four cc. of this 50 per cent blood suspension was injected intravenously into each of a number of donors. After an interval of four months (the maximum survival time for transfused red blood cells) a second intravenous injection was given, and ten days later blood was drawn for testing. Donors with a satisfactory titer of anti-Rh agglutinins were bled.

RESULTS

Nine donors completed the course of immunization. Of these, four showed no evidence of antibody formation while five had anti-Rh agglutinins in their serum. In three of the latter five donors, the serum was too weak (titers from 2 to 12 units) to be useful, but in the other two donors, the titers of anti-Rh agglutinins were very high, 200 and 600 units respectively. By the method of exchange transfusion with type rh donors, 1500 cc. of blood was drawn from each man and a satisfactory yield of testing serum was obtained.

The antibody response was evanescent and the titers soon fell. Six weeks later, each man was given a second provocative injection of Rh-positive blood and this procedure was repeated several times at intervals of six weeks. While each injection again stimulated a rise in the antibody titers, the peaks were not as high as after the first stimulating injections. The donor who originally had the highest agglutinin titer (600 units) subsequently produced blocking antibodies as well as agglutinins, so his serum could no longer be used as a pure agglutinating serum, although it still could be used by the conglutination method,^{10, 11} or by the slide method of Diamond and Abelson.^{2, 3} The second donor with high-titered agglutinins subsequently produced anti-Rh' agglutinins as well as anti-Rh₀ agglutinins. With the aid of potent anti-Rh₀ blocking serum, a useful anti-Rh' agglutinating reagent could be prepared from his serum. Since Rh' is a more potent antigen than Rh'', we have no doubt that by immunizing type rh donors with type Rh₁ blood we shall succeed in obtaining anti-Rh' reagents in the same way. In fact, we are now injecting type Rh₁Rh₁ individuals with type Rh₂Rh₂ blood in the hope of obtaining serum with agglutinins for the factors Hr', Rh'', or both.

SUMMARY

A simple method of preparing anti-Rh₀ agglutinating serum in normal human subjects has been described. Four cc. of a 50 per cent suspension of Rh-positive blood is injected intravenously and a second injection is given four months later. Subjects with satisfactory titers are bled ten to fourteen days following the second injection. Of nine men immunized by this technic, four failed to produce antibodies, three produced antibodies whose titer was too low to be useful, while two produced high-titered anti-Rh agglutinins.

ADDENDUM

Since this manuscript was first prepared, our immunization experiments have been carried much further. Our recent results prove that where the injections are continued for a year, by repeating the injections at six week intervals, five out of six injected men respond by producing antisera useful for Rh testing by the tube or slide method, or for Rh typing by the tube method.

The observations on Rh isoimmunization of normal type rh males are also of great value in throwing light on the mechanism of isosensitization of pregnant women. To cite one example, we have found that the men injected with type Rh₂ blood first produce anti-Rh₀ agglutinins, then anti-Rh₀ glutinins (blocking antibodies), and then anti-Rh" agglutinins. They finally reveal anti-Rh" serums (with natural anti-Rh₀ blocking antibodies), which are useful for providing anti-Rh" reagents for Rh typing. In a similar way, type rh men injected with type Rh₁ blood first produce anti-Rh₀ agglutinins, then anti-Rh₀ glutinins, and then anti-Rh' agglutinins. These observations conform with our previous finding that Rh₀ is a much better antigen than Rh' or Rh", and furnish additional evidence supporting our theory of competition of antigens to explain analogous phenomena observed in connection with isosensitization by pregnancy (Wiener, A. S.: Competition of antigens in isosensitization by pregnancy. *Proc. Soc. Exper. Biol. and Med.*, 58: 133-135, 1945).

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A COMBINED CONCENTRATION METHOD FOR TUBERCLE BACILLI*

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A concentration method for tubercle bacilli has been developed which employs certain desirable features of two methods now in use but eliminates some objectionable feature of each method. It has proved to be consistently successful over a period of two years, and in this laboratory, has almost superseded preparations of direct smears. It avoids difficulties in applying material to the slide, in decolorization, and in handling tenacious sputum.

One modification of Petroff's original concentration method utilizes a digestion of the sputum or other body fluid by 0.5 per cent sodium hydroxide, with incubation for thirty minutes at 37C. The digested mixture is then shaken for ten minutes with a suitable hydrocarbon, such as xylol. The tubercle bacilli, by virtue of their waxy capsules, then float to the surface of the digested material. After standing for ten to fifteen minutes, the xylol containing the tubercle bacilli is picked from the surface with a platinum loop, transferred to a slide and stained. This procedure is time-consuming and laborious. In many instances, the sodium hydroxide fails to digest the sputum completely with the result that the xylol cannot penetrate or contact all of the mixture. When this occurs, additional sodium hydroxide must be added and the material again incubated before the mixture can be shaken with xylol.

The method of Oliver and Reusser¹ employs commercial chlorox (Chlorox Chemical Co., Oakland, Calif.), which is a slightly alkaline solution consisting of equal parts of 15 per cent sodium hydroxide and 20 per cent sodium hypochlorite. The sputum or other body fluid is mixed with an equal volume of chlorox and shaken for two or three minutes. The mixture is kept at room temperature for ten minutes, then centrifuged at 3000 r. p. m. for ten minutes. The sediment is transferred to a slide and stained. This method is rapid and the material is sterilized effectively by the chlorox. Once applied to the slide, however, the sediment is difficult to decolorize and retain on the slide, even with the aid of albumin-glycerol. This results in preparations that are untidy and difficult to read.

METHOD

Sputum or other body fluid is mixed with an equal volume of chlorox and shaken for two or three minutes. One or 2 cc. of xylol is added and shaken with the digested material, for from five to ten minutes. The mixture is allowed to stand at room temperature for ten minutes. The xylol is ladled from the surface of the material, transferred to a slide, dried and stained.

* Received for publication, August 15, 1946.

SUMMARY

A method has been described which combines the better features, and eliminates certain objectionable features, of two widely used methods of concentrating tubercle bacilli. The combined method is simple, rapid, and produces uniformly satisfactory results.

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STAINING TUBERCLE BACILLI IN TISSUE AND SMEAR, EMPLOYING AEROSOL O. T. OR SODIUM LAURYL SULFATE*

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The following modification of the Ziehl-Neelsen stain has been found to be rapid and efficient in demonstrating tubercle bacilli in tissue and smears. No heat processes are involved and the total time consumed in preparing the slide amounts to about eight minutes. The counterstain used, Light Green SF Yellowish,† does not fade out and has been found to be a relatively permanent stain. The modified stain can be utilized in preparing 25 slides at the same time and is not as time-consuming as the original stain.

METHOD

1. Deparaffinize sections in the usual manner.
2. Wash thoroughly in cold running water.
3. Stain three minutes in carbol fuchsin containing two drops of Aerosol O. T.,‡ 8 per cent aqueous solution or sodium lauryl sulfate,‡ 2 per cent in each 50 cc. of stain. The Aerosol or sodium lauryl sulfate should be added to the stain just before use.
4. Wash in cold water.
5. Decolorize in 95 per cent alcohol containing 2 per cent hydrochloric acid until pale pink.
6. Wash in cold water.
7. Stain with Light Green SF Yellowish (two minutes).
8. Dehydrate in absolute alcohol (about two minutes).
9. Dehydrate in absolute alcohol (two minutes).
10. Clear in methyl salicylate (two minutes).
11. Clear in xylol (two minutes).
12. Clear in xylol (two minutes).
13. Mount in Canada balsam.

REAGENTS†

1. Aqueous solution Aerosol O. T., 8 per cent.
2. Aqueous solution sodium lauryl sulfate.
3. Stock solution phenol, 5 per cent aqueous.
4. Stock solution basic fuchsin, 10 per cent in 95 per cent alcohol.

* Received for publication, September 27, 1946.

† Aerosol O. T. may be obtained from the American Cyanamide and Chemical Company, New York, N. Y., sodium lauryl sulfate from the E. I. DuPont de Nemours and Company, Inc., Wilmington, Del., and Light Green SF Yellowish from the Pharmaceutical Laboratories, National Aniline and Chemical Company, Inc., New York, N. Y.

5. Stock solution Light Green:

Light Green SF Yellowish.....	2 gm.
Glacial acetic acid.....	1 cc.
Distilled water, q.s. ad.....	100 cc.

STAINING SOLUTIONS

1. Light Green counterstain:

Stock solution.....	10 cc.
Glacial acetic acid.....	2 cc.
Distilled water, q.s. ad.....	100 cc.

2. Carbol fuchsin stain:

Stock solution basic fuchsin.....	10 cc.
5 per cent phenol, aqueous, q.s. ad.....	100 cc.

RAPID METHOD OF STAINING TUBERCLE BACILLI WITH TERGITOL #7*

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The use of Tergitol #7† in the rapid staining of the tubercle bacillus has been reported by Muller and Chermock.¹ According to these authors, the method described eliminates the use of heat, shortens the staining time, and gives equal or better results than the older methods without loss of specificity for the acid-fast organisms.

Our method for staining direct smears is practically identical with the one reported, but our method for staining tissue sections is considerably shorter. The entire process of removing the paraffin, staining, clearing and mounting can be completed in less than ten minutes. This is approximately twenty-six minutes shorter than the method described by Muller and Chermock. Because of its simplicity and the gratifying results, we believe the method is worth reporting.

METHOD

Our technic in staining direct smears differs from that of Muller and Chermock only in the manner of applying Tergitol #7 to the stain. The smears are fixed with heat in the usual manner and the slide is covered with Kinyoun's carbol fuchsin (phenol, 8 gm.; fuchsin, 4 gm.; 95 per cent alcohol, 20 cc.; water, 80 cc.). One drop of Tergitol #7 is added and the cold stain is allowed to remain for one minute. The smear is then decolorized in 3 per cent acid alcohol and counter-stained with 3 per cent methylene blue for one minute (methylene blue, 3 gm.; 95 per cent alcohol, 20 cc.; water, 80 cc.).

1. Our method for staining tissue sections is as follows: Fix the tissue in 10 per cent neutral formalin and dehydrate and clear in successive alcohols and xylols in the usual manner. Embed in paraffin and cut sections from 5 to 10 microns in thickness.

2. Remove the paraffin by placing in xylol for one minute, absolute alcohol for one minute and 95 per cent alcohol for one minute.

3. Flood the slide with Kinyoun's carbol fuchsin.

4. Add one drop of Tergitol #7 and stain without application of heat for one minute.

5. Wash in water.

6. Decolorize in acid alcohol until the tissue appears clear. This requires two or three washings (ten to fifteen seconds).

* Received for publication, October 23, 1946.

† This material was obtained from the Carbide and Carbon Chemicals Corporation, Indianapolis, Indiana. "Tergitol" wetting agent 7 is a 25 per cent aqueous solution of the sodium sulfate derivative of 3, 9-diethyltridecanol—6.

7. Counterstain with 3 percent methylene blue for one minute. (Do not use potassium hydroxide in the methylene blue as it gives a blurred counterstain in tissues).

8. Wash in water.

9. Dehydrate in 95 per cent alcohol for one minute and acetone for one minute. Clear in xylol for one minute. Mount in clarite or balsam immediately.

RESULTS

The acid-fast organisms stand out as large, brilliant red, often beaded bacilli against a clear blue background. The bacilli appear much larger than they do when stained by ordinary Ziehl-Neelsen, or Gabbett's stains. They are readily seen under high power, so we now use the high dry lens for routine work. Many more organisms are seen with this method than with any of many modifications of the carbol fuchsin stain that we have used. As is usual with tissue sections, most of the acid-fast bacilli are seen in caseous areas. A few are seen in giant cells.

The counterstain is excellent. The nuclei of the cells are large, clear, and sharply outlined. The cytoplasm is pale, but distinct. The giant cells are large and clear. Red blood cells take a pink-orange color, but they cannot be confused with the tubercle bacilli. As long as the time intervals given in step number 2 are observed, there is no precipitation of stain and there are no red areas, as are often seen in other methods.

DISCUSSION

We have been able to demonstrate the organisms with as little as five seconds exposure to the stain, but the number of organisms seen is reduced and many are incompletely stained. With exposure to the stain for one-half minute, the results are excellent, but we have established a routine time of one minute to reduce the possibility of error.

We experimented with a method of mixing the carbol fuchsin and Tergitol in advance, using one drop to 25 cc. of stain. (Muller and Chermock used one drop to 30 to 40 cc.). This method was successful, but the stain begins to lose its strength in approximately one week. The procedure is practical only if the stain is used within six or seven days after it is made.

The rapid Tergitol #7 stain has been consistently positive in known positive cases with acid-fast bacilli and consistently and unequivocally negative in negative controls. In all cases, the number of organisms seen has been far greater than with the standard methods of steaming, or cold staining with carbol fuchsin for varying periods of time. We have obtained positive readings in histologically positive and histologically doubtful tissues, when a number of other commonly accepted carbol fuchsin stains were all negative. We have not compared this method with the fluorescent methods.

SUMMARY

The method for staining acid-fast bacilli, using Tergitol #7 instead of heat as a penetrating agent, has been employed and found to be simple, short and reliable. A new method of employing this agent in tissue sections, which shortens the staining time and gives excellent results, has been described. This method permits the use of the high dry objective for routine examination of acid-fast bacilli.

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STAINING OF BACTERIA IN PARAFFIN SECTIONS

LILLIE-GRAM STAIN FOR BACTERIA AND FLEXNER STAIN FOR LEPROSY BACILLI*

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Lillie² suggested that heating of the crystal violet may be required in the Gram stain, in order to demonstrate some bacteria. In the method here described, this variation in technic is extended to apply to other bacteria including Flexner's stain for leprosy bacilli in tissue sections. Tubercle and leprosy bacilli and fungi stain remarkably well with the Lillie-Gram stain, and leprosy bacilli very easily and quickly with the Flexner stain. Any fixation may be used.

I. LILLIE-GRAM STAIN FOR BACTERIA

Solutions

Crystal violet. Crystal violet, certified, 2 gm.; alcohol, ethyl, U.S.P., 95 per cent, 20.0 ml.; 1 per cent ammonium oxalate, c.p., in all-glass distilled water, 80.0 ml. Place into chemically clean glass-stoppered bottle, cap with paper, label and date. The crystal violet is dissolved in the alcohol at the time the solution is made. Most dyes that have stood in an alcoholic solution for some months precipitate and lose some of their staining properties. The stain is filtered as it is replaced in the dropping bottle. This solution is quite stable and 500 ml. is adequate for routine work.

Lugol's solution (formula for the Gram and Flexner stains for leprosy bacilli only). Iodine crystals, c.p., 1.0 gm.; potassium iodide, c.p., 2.0 gm.; tap water, 100.0 ml. Add 3.0 gm. of sodium bicarbonate to each 100.0 ml. of Lugol's solution to alkalize the solution to prevent the bacilli from decolorizing.

Lugol's solution (formula for routine use). Iodine crystals, c.p., 1.0 gm.; potassium iodide, c.p., 2.0 gm.; water, distilled, 100.0 ml.; add 0.2 ml. of a saturated solution of sodium bicarbonate made in tap water to each 100.0 ml. of Lugol's solution. It is well to have two bottles for each formula so that there will be a batch ready when needed. The same is convenient for the crystal violet. The bicarbonate causes the section to retain the blue stain, but does not require as much when the crystal violet has aged for some time. Two volumes of 250.0 ml. each is adequate for routine work.

Safranin. Safranin O, certified, 0.5 gm.; all-glass distilled water, 100.0 ml. Place into a chemically clean glass-stoppered bottle, cap with paper, label and date. A volume of 250.0 ml. is adequate for routine work. A 0.25 per cent aqueous solution is used for contrast staining only.

Acetone. Acetone c.p. or analytical quality.

The sections may be cut from 5 to 8 or more microns in thickness.

1. Remove paraffin and precipitates, if necessary, and bring to water.
2. Hold the slide with a pair of forceps and apply the crystal violet with a dropping bottle, preferably with a medicine dropper. Pass back and forth over the flame of a micro-burner about five times, or until the solution begins to steam; then let cool for five seconds.
3. Without washing, apply the Lugol's solution from a dropping bottle to the slide, washing the crystal violet off with it.
4. Without washing, apply the acetone from a dropping bottle, to decolorize superficially and wash in water. Apply more Lugol's solution, for five seconds, to the film of

* Received for publication, June 22, 1946.

crystal violet left on the section and decolorize again with acetone and wash in water. If the section retains much of the blue stain, apply the Lugol's solution longer, decolorize and wash again. The section should be cleared of the blue color each time before repeating the staining procedure.

5. Repeat steps 2, 3 and 4 two more times. After three times, the sections will have been stained completely. Examine under the low power of the microscope. If the nuclei and other elements retain the blue stain, apply more Lugol's solution to the section for one-half minute, and apply more acetone and wash in water. The bacteria will not decolorize. As the user of this stain becomes familiar with it, he will find it easy to control the decolorization without repeated application of the Lugol's solution to remove the excess stain.

6. Apply safranin from a dropping bottle, for from one-half to five minutes, depending on whether gram-negative bacteria are to be demonstrated. One-half minute is required for contrast staining only, five minutes for staining gram-negative bacteria. Wash in water.

7. Apply Lugol's solution to the stain evenly and quickly and wash in water.

8. Dehydrate with acetone and clear with xylol. Clean the slide around the section and on the under surface and repeat the dehydrating process with acetone, and the clearing with xylol. It is well to hold the slide in a horizontal position when applying the acetone, and to tilt the slide slightly to apply the xylol in such a manner as to wash the acetone off the slide with the xylol. The procedure should be done quickly.

9. Mount in clarite or similar mounting medium.

RESULTS

The gram-positive organisms are blue-black; gram-negative organisms, red; nuclei, clearly red with a pink background.

DISCUSSION

In the application of heat in the staining of organisms in tissues, it was found that repeating the staining with crystal violet and Lugol's solution with subsequent decolorization with acetone insures staining and enhances the demonstration of morphologic characteristics of gram-positive organisms. Gram-negative organisms may be stained by leaving a film of Lugol's solution on the slide after the last application of crystal violet, washing the slide in water, applying safranin for fifteen seconds, then acetone, and quickly following with a rapid application of xylol. With the film of Lugol's solution on the section, the safranin is prevented from staining the section diffusely, and it stains only the elements for which it has the greatest affinity.

II. FLEXNER STAIN FOR LEPROSIS BACILLI

Solutions

Ziehl-Neelsen's carbol fuchsin. Basic fuchsin, certified, 0.6 gm. dissolved in 10.0 ml. of 95 per cent ethyl alcohol, U.S.P.; 5 per cent carbolic acid in all-glass distilled water, 90.0 ml. Filter (not essential) into a 100.0 ml. chemically clean dropping bottle; label and date. The basic fuchsin dye is dissolved at the time the stain is made up. Do not prepare more stain than that required for a period of two months, as after that time, the stain should be discarded. Freshness of this stain is essential for success. If a stronger stain is desired, 0.2 gm. of basic fuchsin dye may be added to 100.0 ml. of carbol fuchsin after it is made up; this stronger stain is better for the regular acid-fast method, but not for the Flexner stain, since it is more difficult to decolorize.

Lugol's solution. The formula is the same as that used in the Lillie-Gram method. It is believed essential, however, to add sodium bicarbonate to this solution and that it be made with tap water.

Toluidine blue O. Toluidine blue O, certified, 0.5 gm.; 20 per cent ethyl alcohol, U.S.P., in all-glass distilled water; 0.5 ml. of glacial acetic acid.

Acid alcohol. Hydrochloric acid, c.p., concentrated, 1.0 ml.; 70 per cent ethyl alcohol, U.S.P., 99.0 ml.

Acetone. Acetone c.p. or analytical.

1-5. The technic is similar to steps 1 to 5 of the Lillie-Gram stain given above, except that in step 2, instead of crystal violet, Ziehl-Neelsen's carbol fuchsin is used and, after the staining begins, a few drops of cold carbol fuchsin are added, and the slide is allowed to stand one minute.

6. Apply the toluidine blue O-acetic acid solution for fifteen seconds.

7. Do not wash; dehydrate with acetone; clear with xylol. Clean the slide around the section and under surface, apply acetone and xylol again. Mount in clarite, or similar mounting medium.

8. Before applying the counterstain, acid alcohol may be applied to the section to test for acid-fastness or to remove more of the carbol fuchsin if desired; wash in water.

RESULTS

Lepra bacilli are brilliant red and other bacteria are blue. Nuclei are blue with a lighter blue background. Gram-negative bacteria show clearly with this stain.

DISCUSSION

The lepra bacilli stain distinctly with one application of stain. It is well to run test slides of each series of sections to determine the technic preferred. The carbol fuchsin may be applied once for five minutes after the preliminary heating and the sections decolorized as in steps 3 and 4. Repeated heating of the carbol fuchsin causes the section to retain the stain so that it is necessary to apply more of the Lugol's solution and acetone with washing in water to obtain adequate decolorization. Test slides may be made with the decolorizing step to determine the depth of color of carbol fuchsin over which the toluidine blue will stain. Even though the color of the carbol fuchsin is rather intense, the toluidine blue replaces most of it and the lepra bacilli are stained more intensely.

An acidified toluidine blue O was used because acetone, in dehydrating, does not differentiate the stain as does ethyl alcohol.

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AN ADAPTER FOR BACTERIAL FILTERS*

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The sterile preparation of serums and other thermolabile solutions requires passage through a Berkefeld candle, Seitz, or sintered glass filter. Usually, the

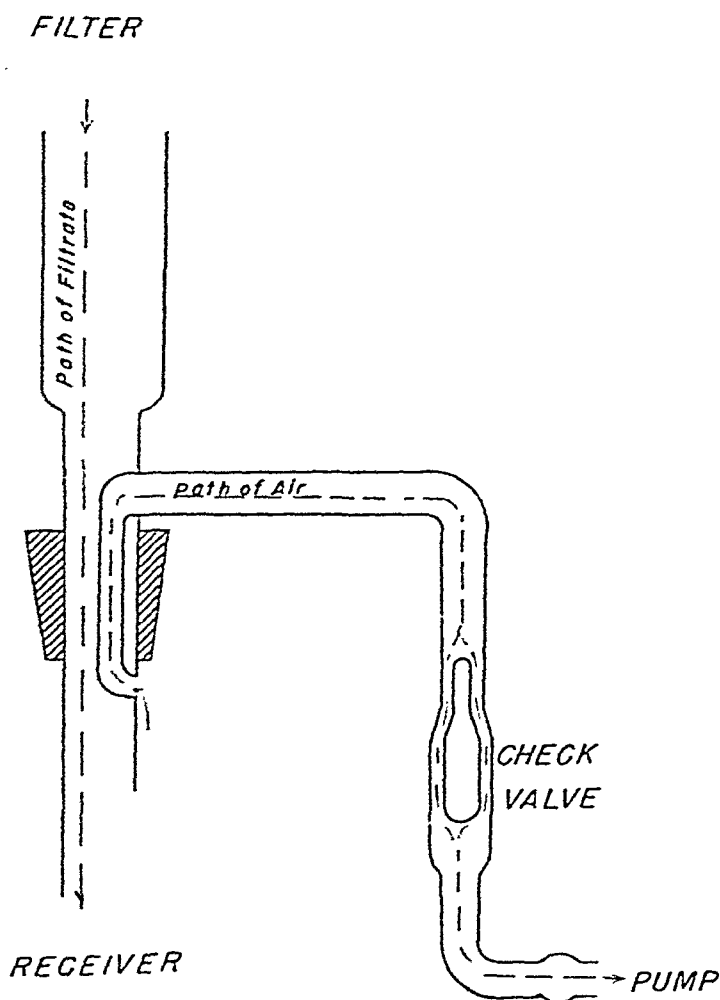


FIG. 1. DIAGRAM OF ADAPTER SHOWING ARRANGEMENT OF INNER TUBE

mechanism that is set up in the laboratory demands the close attention of the operator until the very slow filtration is completed. This is inefficient, since it requires the services of a highly trained technician for a long time. When sizable amounts of solution are to be prepared, entire working days may be consumed.

In order to obviate this costly procedure, an adapter was devised to carry out the filtration with a minimum of attention. Adapters now in use are faulty in that the filtrate passes through the delivery tube countercurrent to the air which

* Received for publication, August 14, 1946.

is being exhausted from the receiver. With serum and other viscous solutions, there is undesirable frothing in the adapter and significant amounts of the solution are entrained in the air stream and are swept into the tube connected with the pump. Suction must be maintained throughout the filtration, since failure in water pressure might result in contamination of the filtrate with water returned from the pump. The apparatus to be described avoids all these objections.

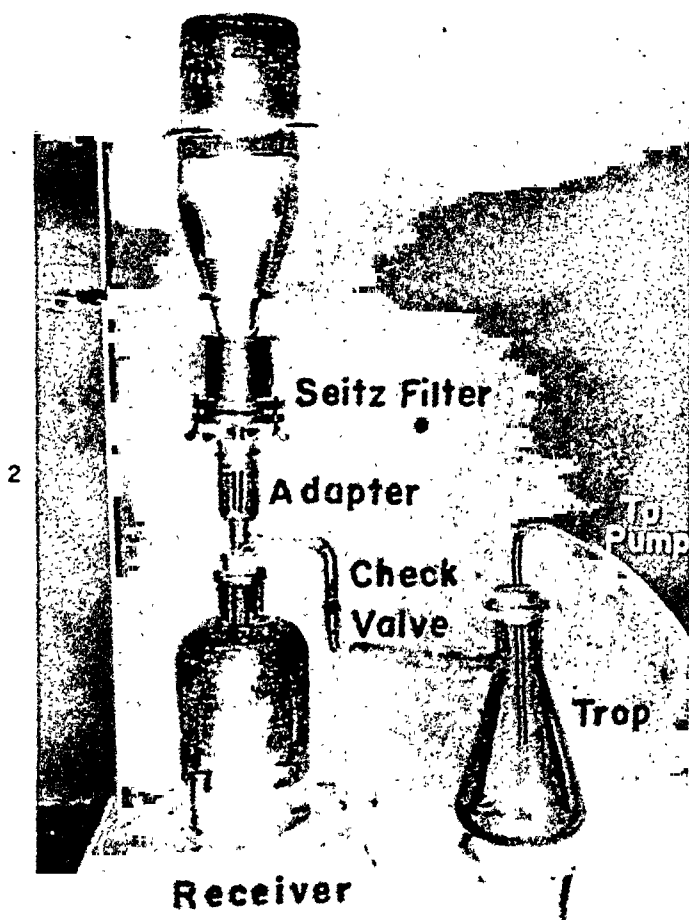


FIG. 2. ADAPTER IN POSITION IN ASSEMBLED APPARATUS

The adapter (prepared by Eimer and Amend of New York City), shown in Figures 1 and 2, consists of a Pyrex glass tube, one end of which measures 25 mm. in diameter, while the other measures 15 mm. in diameter. The Seitz filter is fitted through a No. 6 rubber stopper into the wider end of the adapter, while the narrower end passes through a rubber stopper into the mouth of a receiving bottle. The narrower end of the adapter contains an inner glass tube. One end of this inner tube terminates as a hole in the wall of the outer tube where it communicates with the interior of the receiving bottle just beneath the rubber stopper. Just above the rubber stopper outside of the receiving bottle, the inner tube passes at right angles through the sealed wall of the outer tube. The inner tube

then continues as a side tube, and communicates with a trap by means of a connecting piece of rubber pressure hose. A check valve is fitted into the side tube between the receiving bottle and the rubber hose (Fig. 2). When the air is exhausted from the receiving bottle, it leaves the latter through the inner tube and so avoids contact with the filtrate coming down through the outer tube of the adapter.

The Seitz filter is inserted into the mouth of the adapter through a rubber stopper; a piece of pressure tubing is connected to the end of the side tube, and the apparatus is then sterilized in the autoclave. The free end of the pressure tube is connected to a filtering flask which serves as a water trap and leads to the pump. The solution to be filtered is placed in a container having the same capacity as the receiving bottle. This container is supported with its neck down and its mouth is inserted into the Seitz filter. It is convenient to have the mouth of the container fitted with a screw cap. This is first loosened so that it is held by but one turn of the thread and the cap is removed after the bottle is in place (Fig. 2). The suction is turned on for about fifteen minutes and then suddenly turned off. The check valve closes immediately, maintaining the vacuum in the receiver. Any water that might be aspirated from the pump is caught in the trap and the rest of the apparatus is protected against contamination. The fluid is drawn into the receiving bottle and, as the level in the mouth of the Seitz filter falls, the water seal at the mouth of the container is broken and more of the fluid flows into the filter until the seal is re-established. This alternating process then continues until the vacuum is broken after all of the liquid has left the bottle. Since no active pumping is necessary once the vacuum has been established, the apparatus requires no further attention and may be permitted to run overnight. Usually, the entire contents of the bottle will pass into the receiver during this period and all the operator has to do in the morning is to remove the adapter from the receiver and replace it with a sterile stopper.

STAINING OF ELASTIC TISSUE BY FUCHSIN-SULFUROUS ACID

VARIABILITY IN DIFFERENT SPECIES*

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This paper reports a difference in staining property of elastic tissue of different species of animals to fuchsin-sulfurous acid or Schiff's reagent (basic fuchsin reduced by sulfurous acid).

Reduced fuchsin reacts with aldehydes in the test tube and in tissue sections to form a magenta colored compound.¹ In Feulgen's method for staining nuclei of cells, the sections are first hydrolyzed with hydrochloric acid. This treatment releases a pentose from thymonucleic acid in the nuclei and the pentose reacts with Schiff's reagent. Bauer's method for staining glycogen and other polysaccharides consists of treating the tissue or sections with chromic acid and then staining the oxidized products with Schiff's compound. Evidently, reduced basic fuchsin is a valuable reagent for histochemical studies on sections of tissue.

Although little is known about the chemical constitution of elastic tissue, two chemical properties have been discovered and found useful.⁴ These properties are: (1) its resistance to the action of acid; and (2) its capacity to reduce ferric chloride and other compounds.

The customary methods of staining elastic tissue are by Weigert's resorcin-fuchsin and by Taenzer-Unna's orcein stain.⁴ By these methods, a difference in staining of elastic tissue has been observed in different species of animals. Lower animals have an elastic-like tissue which does not take Weigert's stain. In higher vertebrates and in man, the elastic fibers can be stained by both methods.

While working with Feulgen's stain on rabbit tissue in this laboratory, it was observed that the elastic fibers in arterioles were stained. It was thought that this method would be useful for routine pathologic work but, unfortunately, human elastic tissue is not stained by the reduced fuchsin.

On reviewing the literature, it was found that Feulgen and Voit,³ Voss,⁷ Odiette⁶ and Bujard² have reported on the staining of elastic fibers by Schiff's reagent. Voss also reported that Feulgen's method stained the elastic tissue of the rabbit, sheep, cow and horse, but not that of man, dog and cat.

METHOD

The method is similar to those described by McClung⁵ for staining sections of tissue for thymonucleoprotein. The tissue is fixed from twelve to twenty-four

* Published with the permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or the conclusions drawn by the authors. Received for publication, July 2, 1946.

hours in acidified mercuric chloride (6 per cent mercuric chloride, 98 parts; glacial acetic acid, 2 parts). It is then imbedded in paraffin and sectioned. The slides are carried through xylol, alcohol, water, Lugol's solution and 5 per cent sodium thiosulfate. One section is hydrolyzed for five minutes in N/1 hydrochloric acid at 60 C. and then stained from sixty to ninety minutes in Schiff's reagent (0.5 per cent basic fuchsin, 200 cc.; N/1 hydrochloric acid, 20 cc.; sodium sulfite, 1 gm.). Another section is stained directly without preliminary hydrolysis. The excess reagent is washed three times with sulfurous acid (10 per cent sodium sulfite, 10 cc.; N/1 hydrochloric acid, 10 cc.; water, 200 cc.) and then in water. If desired, the sections may then be counterstained with fast green FCF (0.5 per cent in 95 per cent ethyl alcohol).

OBSERVATIONS

Sections of aorta, spleen, lung and other organs of the rabbit and rat were treated by the above methods. Fibers, which were apparently elastic fibers, stained purple, both in the sections subjected to hydrolysis and in those not hydrolyzed. Figure 1 shows the layers of elastic tissue in the intima and media of the aorta of a rabbit. In Figure 2, the spleen of a rat is seen to have many delicate and coarse fibers in the capsule and trabeculae. In the alveolar walls of the lung, delicate fibrils can be demonstrated by Schiff's reagent. The arterioles in sections of various organs have a layer of elastic fibers in the intima. The reduced fuchsin was found to stain the elastic fibers in tissue derived from the rat, rabbit, guinea pig, chicken and cow.

Elastic fibers in tissue obtained from human postmortem and operative material failed to stain with Schiff's reagent with, or without, preliminary hydrolysis. The nuclei of the cells, however, stained well with the reagent after hydrolysis. In Figure 3, an artery from a man's amputated arm shows nuclei which are well stained with the reagent, but the internal and external elastic membranes and a layer of elastic tissue in the media are not stained.

DISCUSSION

In general, Schiff's reagent is not reliable for the demonstration of elastic fibers, since even optimal results with this reagent are inferior to those obtained with the stains routinely used for elastic tissue. The fuchsin-sulfurous acid is, however, useful in studying the chemistry of elastic tissue. The present observations raise two questions: What is the compound in elastic tissue which reacts with Schiff's reagent? In what way does the elastic fibers of man differ from those of the animals tested? Our present knowledge of the chemistry of elastic tissue seems inadequate to answer these questions.

SUMMARY

Fuchsin-sulfurous acid, or Schiff's reagent, readily stained the elastic tissue of the rabbit, rat, guinea pig, chicken and cow, but not the elastic tissue of man.

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FIG. 1. AORTA OF RABBIT, STAINED WITH FUCHSIN-SULFUROUS ACID AFTER
HYDROLYSIS WITH HYDROCHLORIC ACID

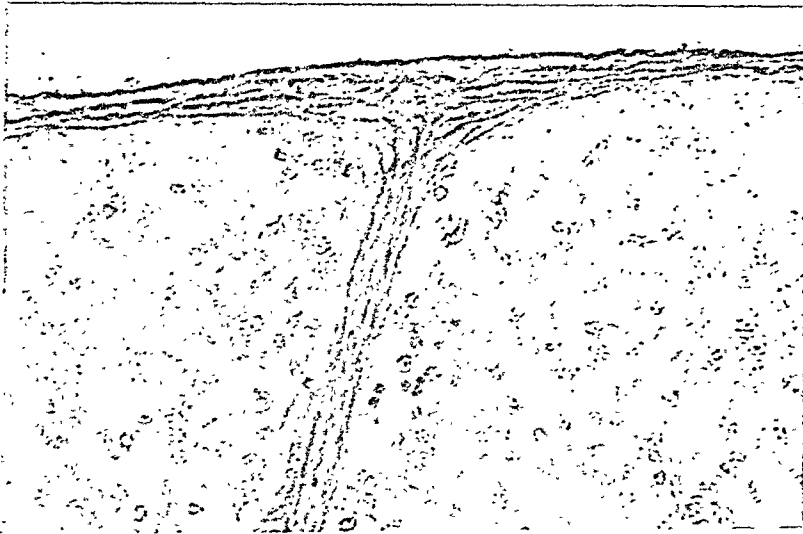
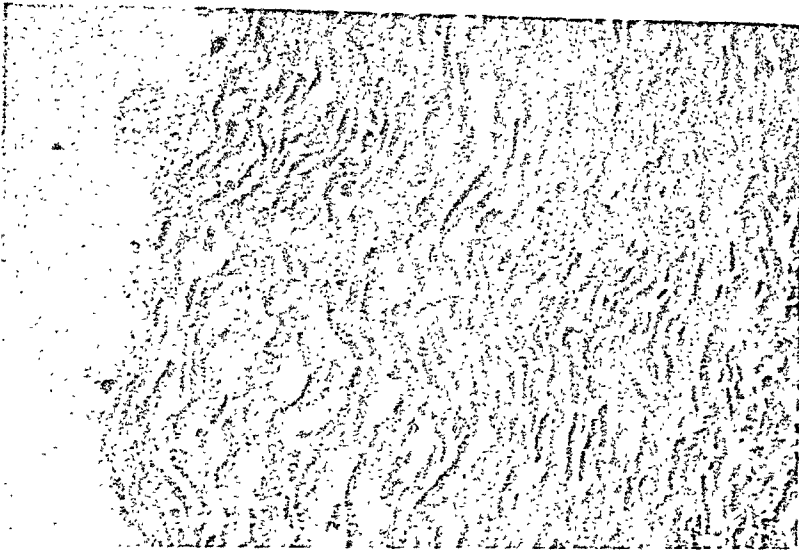
The elastic tissue in the intima and media and the nuclei of the smooth muscle cells are well stained. $\times 450$.

FIG. 2. SPLEEN OF RAT, STAINED WITH FUCHSIN-SULFUROUS ACID WITHOUT
PRELIMINARY HYDROLYSIS

Elastic fibers in the capsule and trabeculae are stained. The nuclei of the splenic cells are unstained in the section, although in the photograph some nuclei appear dark. $\times 450$.

FIG. 3. BRACHIAL ARTERY OF MAN, STAINED WITH FUCHSIN-SULFUROUS ACID AFTER
HYDROLYSIS WITH HYDROCHLORIC ACID

Nuclei of cells in the intima and media are well stained. Two layers of elastic tissues are translucent and are not stained. $\times 450$.



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A SIMPLE RAPID METHOD FOR ASSAY OF BACTERICIDAL AND BACTERIOSTATIC AGENTS

WITH TECHNIQS FOR ASSAY OF PENICILLIN, STREPTOMYCIN, NEOARSPHENAMINE, MAPHARSEN AND OTHER TRIVALENT ORGANIC ARSENICALS*

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Numerous methods for the assay of penicillin have been suggested, and some have been modified.^{2, 4, 6, 7, 9-14, 16, 17, 22, 23, 25, 26, 28-31} The very multiplicity of these methods and the complexity of the modifications indicate that none of them is entirely satisfactory. The greatest difficulty lies in the fact that results by all of them are grossly inaccurate. The best methods give an accuracy of plus or minus 20 per cent when many replicate determinations are averaged and many of the micromethods and serial dilution-technics give errors of plus or minus 100 per cent. Furthermore, all require time consuming manipulations and most of them require the determination of the activity of several known concentrations of penicillin for each unknown studied.

The technic outlined in this article is the result of investigations undertaken to obviate some of these difficulties. The principles involved, the mathematic on which they are based, and the modifications of the method for special purposes, as well as more detailed observations on certain phases of penicillin activity are described extensively in another paper.¹⁹

In brief, the principle of the method is based upon two observations. First, if a broth culture of *Staphylococcus aureus* and a penicillin-containing culture of the same organism are observed during a period when their growth curves make a straight line, the tangent of the angle which the line of the penicillin-containing culture makes with the line of the control culture is proportional to the concentration of the penicillin. Second, after an interval of five to thirty hours, the difference in turbidities between the control and the penicillin-containing culture will be proportional to the square root of the concentration of the penicillin.

These two facts make it possible to check results by two methods, or either may be used alone. Such calculations can be simplified by the use of nomographs (Figs. 1 to 4). At the end of two and one-half hours, results calculated by method 1¹⁹ will give an accuracy of plus or minus 10 per cent and, nomographically determined, an accuracy of plus or minus 25 per cent. Earlier

* Aided by a grant from John C. Higgins. The penicillin and streptomycin used were made available by the Food and Drug Administration of the Federal Security Agency, E. R. Squibb and Sons, The Upjohn Company and the Abbott Laboratories. The trivalent organic arsenicals were supplied by the Abbott Laboratories. Received for publication, September 30, 1946.

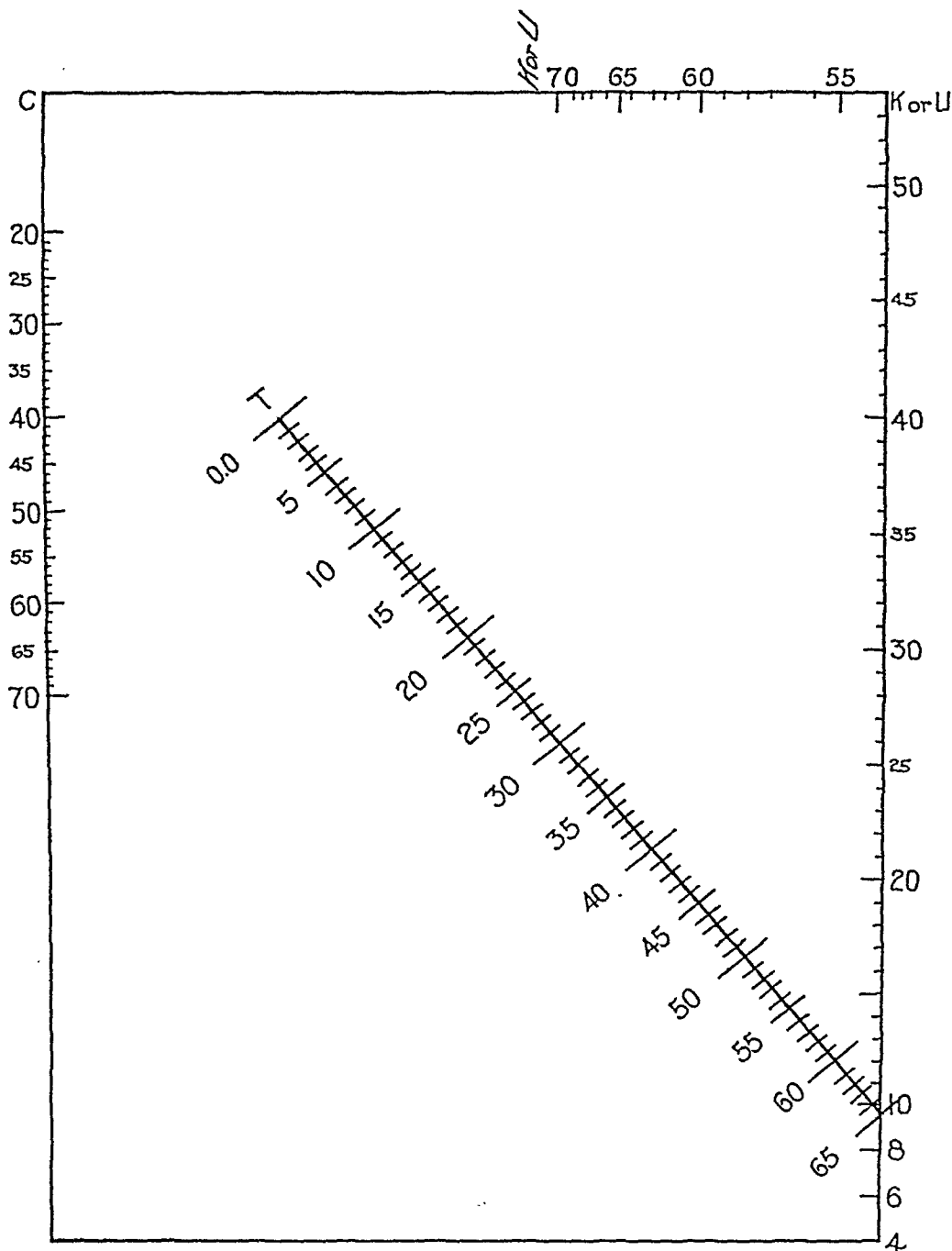


FIG. 1. Nomograph* for use with method 1. Stretch a black thread from the value for *C* in the control culture on the left hand side to the value of *K* or *U* in the culture containing the antibacterial agent, and record the value of *T* at the point of intersection with the diagonal line for the known and each of the unknowns. Use these with nomograph 2. The *T* line may be extended further and marked with equal divisions, if desired.

$T = 100 \times$ the tangent of the angle between the growth curve of the control culture and that of the culture containing the antibacterial agent.

* Additional copies of the nomographs may be obtained from the Arcady Press, 1305 S. W. 12th Street, Portland 5, Oregon.

readings at one and one-half to two hours will give a fair approximation of the penicillin content with an accuracy of plus or minus 35 per cent.

This degree of accuracy is for concentrations of penicillin between 0.01 and 0.06 units per ml. in the final 5.0 ml. used in the test, but plus or minus 100 per cent accuracy is attained at the extremes of the useful range between 0.1 and 0.2 units per ml. and 0.0005 and 0.005 units per ml. It is desirable to dilute the unknowns or to use more than 0.5 ml. of the test fluid when the expected concentration in the undiluted specimen falls outside the range of 0.1 to 0.6 units per ml.

Method 1 gives results within two and one-half hours and may be used with unsterile material. Method 2 is simpler. Both may be done on the same sample with little additional manipulation and somewhat increased accuracy.

No great amount of technical skill or time is required for the test. The photo-electric colorimeter readings require only about ten seconds each. The equipment and materials used are available in most clinical laboratories. Twenty to 40 unknowns may be determined from one control and one known. All transfers are with syringe and needle through vaccine vial caps. Procedures for determinations on blood serum, solutions, urine, and body fluids are given below, but adaptations for testing the penicillin sensitivity of organisms, testing other antibiotics, and for other purposes will be obvious.¹⁹

MATERIALS NEEDED

1. A Klett-Summerson* or other photo-electric colorimeter. A 66 filter is used. This filter transmits red and yellow light well, giving a low blank reading which is little affected by the yellow colors of the medium, of the penicillin solution, and of blood serum, or by the reds in slightly hemolyzed serum.

2. Sets of matched colorimeter tubes. These are cleaned, dry sterilized, and capped with sterile vaccine stoppers. They should all read 0 at the same adjustment when they are filled with distilled water and should all give identical readings when they contain the same solution, reading 150 to 250, such as 1:200 acid hematin¹⁸ read with the 540 filter or a copper sulphate solution read with the 660 filter.

3. A 37 C. water bath, with suitable plastic or wooden racks, as metal racks may scratch the tubes. This should be set near the colorimeter. If method 2 alone is used, an incubator is satisfactory.

4. An ice water bath in the refrigerator for the test culture and the dilute penicillin solutions.

* Obviously, any photo-electric colorimeter may be used. The Klett-Summerson instrument was selected because of the logarithmic scale which obviates the necessity of transposing galvanometer readings to logarithms and because of the convenient size and shape of the tubes. To convert readings in per cent transmission (T) as obtained on other photo-electric colorimeters to the corresponding Klett-Summerson readings (R), use the formula

$$R = (2.000 - \log_{10} T) \times \frac{6,000}{D}, \text{ when } D \text{ equals the internal diameter of the tube in mm.}$$

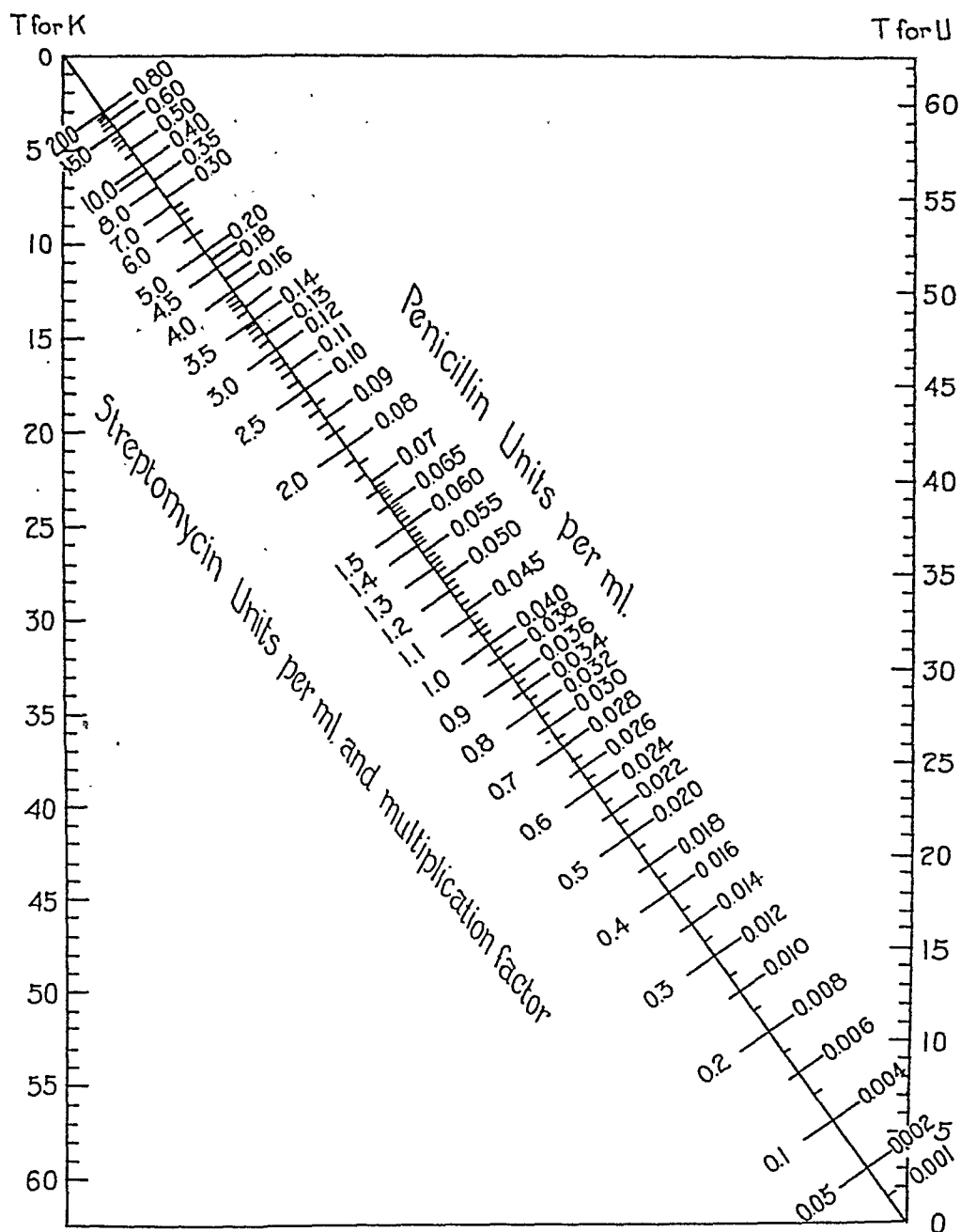


FIG. 2. Nomograph for determining the concentration of antibacterial agents by method 1, from the values of T determined from nomograph 1. Stretch a black thread from the value of T for the known to the value of T for each unknown, and read the concentration of penicillin or streptomycin in the unknowns directly, if the known contains 0.04 units of penicillin or 1.0 unit of streptomycin. If the known contains a different concentration than this or for other antibacterial agents, multiply the concentration in the known by the multiplication factor which is the same as the streptomycin value for 1.0 unit per ml., as read from the nomograph, to get the concentration of the unknown in the broth culture. For example, if a neoarsphenamine known was used, containing 1.5 micrograms per ml., and the thread for the unknown crossed the diagonal line at 2, the unknown contained $2 \times 1.5 = 3$ micrograms per ml. in the final broth culture.

5. A number of Luer syringes of 5, 10 ml. and tuberculin size, and needles of 19 to 23 gauge. The syringes are wrapped in paper and the needles are put in constricted test tubes which are sterilized by dry heat.

6. A 20-gauge needle with cotton plug in the hub. This is sterilized and used to permit egress of air when fluid is being forced into the tubes and vials.

7. A dropping bottle containing 70 per cent alcohol. The rubber stoppers are always punctured through a drop of alcohol.

8. Sterile 0.85 per cent sodium chloride. This is kept in large bottles which are stoppered with vaccine caps. Vaccine vials containing 9, 4, and 3 ml. are prepared from these for making serial dilutions.

9. Standard penicillin solutions. Only one known is necessary, and a final concentration of 0.04 units per ml. will give the best results under the conditions of the test.

The standard can be conveniently prepared from the penicillin the patient is receiving or from standard crystalline sodium penicillin²⁷ which corresponds to 1667 units per mg. Sterile dilutions are prepared in the physiologic saline in the vaccine vials. A stock solution containing 10 units per ml. will keep in the ice bath for about one week. From this, a dilution to contain 0.4 units per ml. is made for use in the test.

10. The test culture. A strain of *Staphylococcus aureus* isolated in this laboratory has given the most satisfactory results by this method. It is a coagulase-positive, strongly hemolytic organism, equally susceptible to F and to G penicillin and is known as the Oregon J strain. It can be obtained by application to the American Type Culture Collection* for number 9801, or the National Collection of Type Cultures† for number 6982. The Oregon J strain will grow to higher turbidities, stay in the straight line phase of growth longer, and give good results with a wider range of penicillin concentrations than either the Oxford H strain or the Food and Drug Administration strain 209 P. These two strains can be used with this method, however, if concentrations of penicillin below 0.01 units per ml. are to be expected and a standard of 0.2, the concentration recommended for the Oregon J strain, is used.

Bacto-tryptose phosphate broth is the culture medium recommended. Hartley broth, yeast broth, ordinary nutrient broth, or the synthetic medium suggested by Rantz and Kirby²⁴ may be used but are less satisfactory.

The broth is prepared according to directions in the Difco Manual and stored in vials containing 25, 50 and 100 ml. amounts, which are capped with rubber vial caps. Each new lot of broth should be read in the photo-electric colorimeter and the results recorded as the broth blank. Readings will usually fall between 6 and 11.

To prepare the culture for use, 5 ml. amounts of a one to three hour broth culture are seeded into 50 to 100 ml. or more of the warmed tryptose-phosphate broth. This is incubated for one to one and one-half hours, at which time 5 ml. is withdrawn and put into a colorimeter tube in the water bath. Readings

* Georgetown University, Washington, D. C.

† The Lister Institute, London, England.

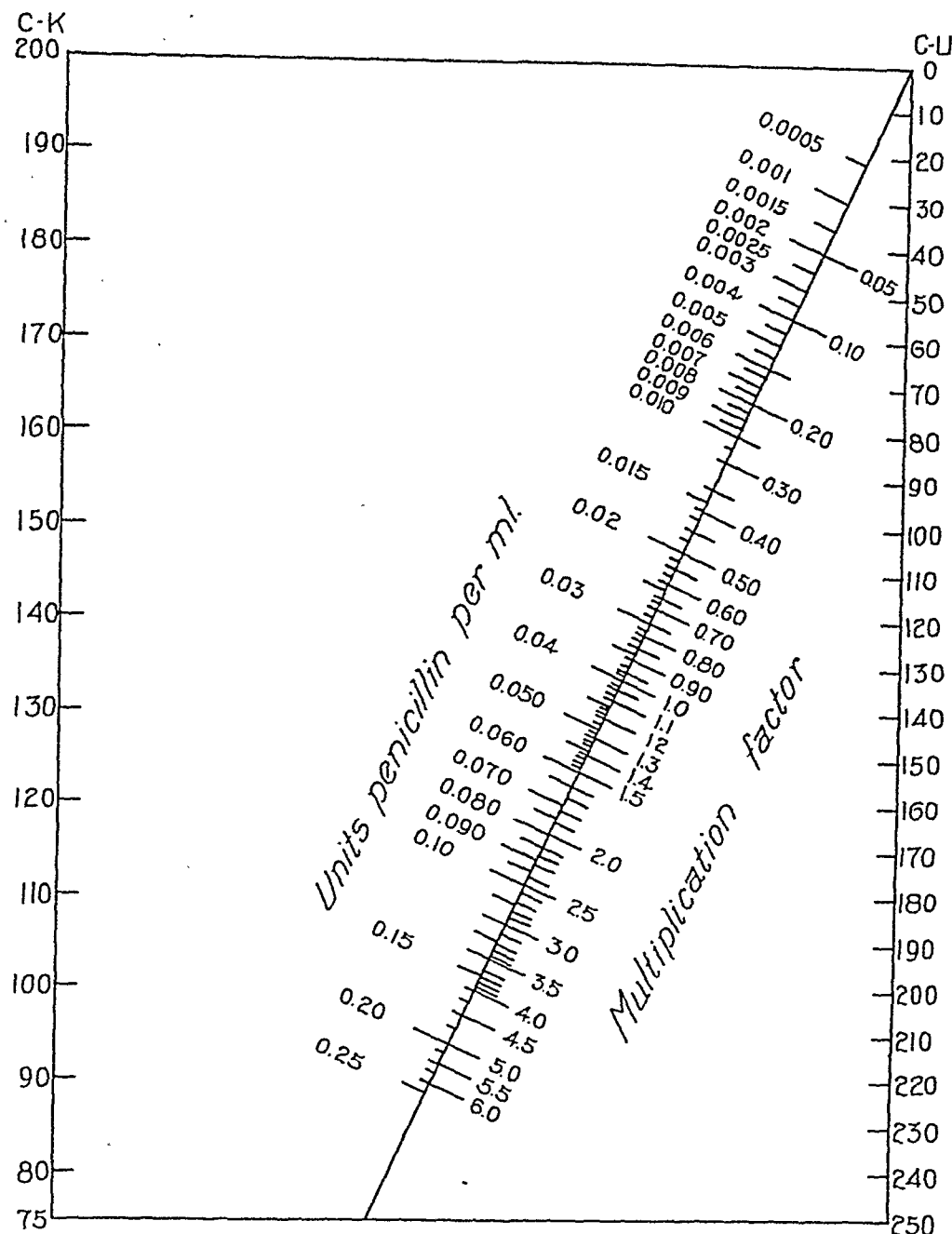


FIG. 3. Nomograph for determination of the penicillin concentration by use of method 2. Read the concentration directly at the intersection of a thread from $C - K$ for the known and $C - U$ for the unknown with the diagonal line, if a 0.04 unit per ml. known was used. If another concentration was used as a known, use the multiplication factor times the concentration of the known. For example, if a known of 0.02 micrograms per ml. of crystalline sodium penicillin was used and the multiplication factor is 3, the unknown contained 3×0.02 or 0.06 micrograms per ml. or 3×0.0333 or 0.1 unit per ml.

are made every ten to fifteen minutes until the reading after subtracting the broth blank is between 12 and 18, ideally 15, corresponding to a colony count of about 300 million per ml. One point on the dial represents a colony count of 20 million

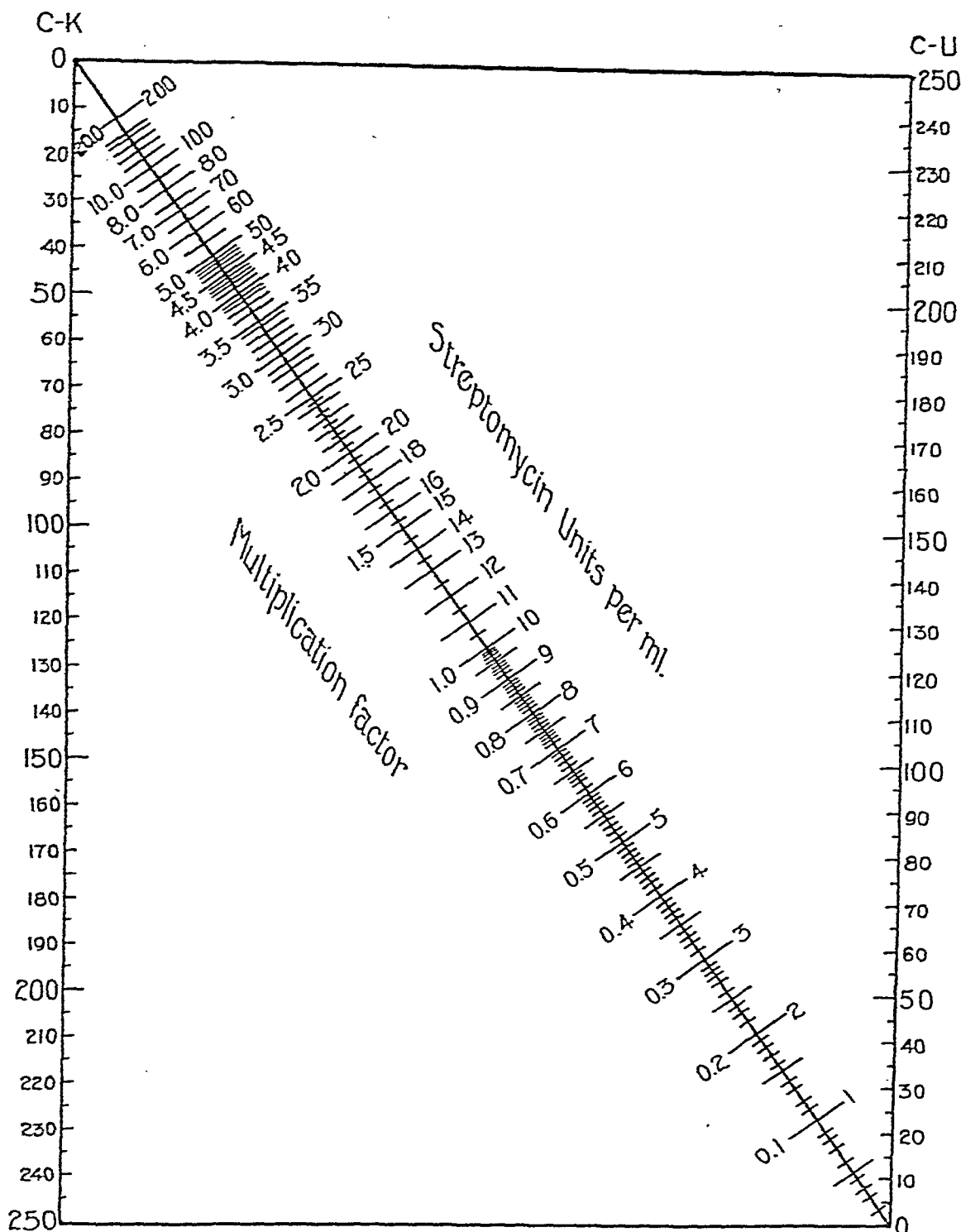


FIG. 4. Nomograph for determination of antibacterial substances other than penicillin by method 2, suitable for streptomycin neomycin, other trivalent organic arsenicals, or antibacterial agents in which reading $C - \text{reading } K$ or U is directly proportional to the concentration. Stretch a thread from the value of $C - K$, for the known, in the left hand column to the value of $C - U$ in the right hand column, and read the concentration of streptomycin directly, if the 10 unit per ml. known was used, as recommended. If a standard of a different strength for streptomycin was used or if testing a trivalent organic arsenical or another antibiotic, use the multiplication factors. Multiply the value of the known \times the multiplication factor for each unknown to get the concentration in the broth culture of that unknown. For example, if a 0.75 microgram per ml. known was used for a mapharsen determination and the multiplication factor for the unknown is 0.2, the concentration of mapharsen in the unknown is 0.2×0.75 or 0.15 micrograms per ml.

per ml. When this reading is obtained* the stock culture is transferred to the ice bath where it will keep for several days without appreciable change. The tube is left in the bath for one hour and reread. If the readings at the beginning and end of the hour do not differ by 30 points or more, make subcultures every two to four hours until rapid growth is reestablished, or try a different lot of Bacto-tryptose phosphate broth.

PROCEDURE

All substances to be tested should be collected, using aseptic technic, refrigerated, and determinations run as soon as practicable. For method 1, unknowns need not be sterile. For method 2, grossly infected unknown material should be sterilized by Seitz filtration. Record the time, preferably thirty to sixty minutes after the last dose of penicillin was administered and, for urine, the time after the last voiding.

Arrange a series of matched sterile colorimeter tubes and number them. Tube 1 is the control, tube 2 the known, and the others are the unknowns.

With a sterile tuberculin syringe, add 0.5 ml. of the control serum† to tubes 1 and 2. Into tube 2, in the same way, introduce 0.5 ml. of the known penicillin in saline containing 0.4 units of penicillin per ml. Into the unknown tubes, introduce equal amounts of the serums to be tested. To each tube, except tube 2, add 0.5 ml. of saline. Using a 5 ml. Luer syringe, add 4.0 ml. of the test culture. Use 1 to 3 ml. of serum and correspondingly less broth culture if a very low concentration is expected.

When both methods are to be used on the same sample, read all tubes in the colorimeter and record readings in tabular form (see examples). This is reading 1, the culture blank. Transfer tubes to the 37°C. water bath and record the time accurately, or set an interval timer for one and one-half hours. The second reading may be made at any time between one and one-half and three hours, but one and one-half hours is most satisfactory. Reset the interval timer for one hour, or that time which has been shown to give an increase in reading of 35 to 50 points, invert the tubes, remove each only so long as is necessary to wipe each dry and read each in rapid sequence. This is reading 2. This should take only about ten seconds a tube. It is essential that each tube be replaced in the water bath as soon as read. At the end of the one hour period, reread each tube rapidly and

* If the culture has increased in turbidity beyond this point and the reaction is still alkaline, it can be used for the test by diluting with *cold* sterile broth until it contains the desired number of organisms. If, for example, the colorimeter reading is 45 after subtraction of the broth blank, dilution of one part of culture with 2.0 parts of cold broth will bring the inoculum to the desired strength. Cultures giving an initial reading of 100 or more will not give satisfactory growth curves even after dilution.

† Control serum may be collected from a healthy individual who is not receiving antibiotics or organic arsenicals,²⁰ or serum collected from the patient before the first dose of penicillin or over 2.5 hours after the last dose may be used. A saline control is satisfactory for most clinical purposes.

in the same sequence.* This is reading 3. Replace the tubes in the water bath or incubator and read after four hours or, for penicillin, the next day. This is reading 4.

When method 1 alone is used, only readings 2 and 3 are necessary. When method 2 alone is used, only readings 1 and 4 are necessary and reading 1 may be omitted if unknowns are not widely different in initial color or turbidity.

For penicillin solutions, use 0.5 ml. of saline in tube 1, 0.5 ml. of the known in tube 2, and 0.5 ml. of diluted unknowns in all other tubes, and add 4.5 ml. of broth culture to each tube.

For urine, dilute the urine to a volume of 200 ml. per minute with sterile 0.85 per cent saline, and use 0.5 ml. amounts. Proceed as for penicillin solutions. *E.g.*, if the interval between voidings was thirty-two minutes and the urine volume 64 ml., dilute 2 ml. to 200 ml. = 0.5 ml. to 50 ml. with 0.85 per cent sodium chloride.

For cerebrospinal fluid, this may contain a high concentration or it may be very dilute, depending on the time which has elapsed. In the former case dilute 1:200 and in the latter case use 1.0 ml. of cerebrospinal fluid and 4.0 ml. of the test culture.

For pleural and ascitic fluids, centrifuge, pass the supernatant fluid through a Seitz filter if grossly purulent, and proceed as for blood serum, using sterile ascitic fluid for the control and known tubes.

NOMOGRAPHIC DETERMINATION OF RESULTS

Method 1. Let C = Reading 3 - Reading 2, for tube 1, the control. This difference should be 30 or over, ideally 35 to 50.

Let K = Reading 3 - Reading 2, for tube 2, the known.

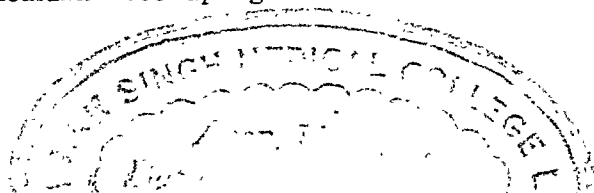
Let U = Reading 3 - Reading 2, for each unknown.

Read T on the diagonal line for the known and each unknown from nomograph 1 at the point of intersection of a thread or straight edge from C on the left hand side with K or U on the right hand side. Read units of penicillin per ml. of culture for each unknown from nomograph 2 at the point of intersection on the diagonal line of a thread from the T reading for the known to that of the unknown. Multiply this value for the concentration in the tube as read by the dilution factor, usually 10 if each 0.5 ml. of unknown was diluted to 5.0 ml., and by any preceding dilution of the unknowns, to get the concentration in the original sample.

Nomographic determination somewhat reduces the accuracy of results by method 1, since nomograph 1 is an approximation. The method of mathematical calculation is given in reference 19.

Example, method 1:

* The closer the difference between readings 2 and 3 for the control tube is to 40, the more accurate the results; so if this is under 35 or over 50, the one hour time interval may be increased or decreased for that lot of medium. Too rapid growth is better than too slow.



READING	CONTROL TUBE 1	KNOWN TUBE 2	TUBE 3	TUBE 4
3 at 2.5 hours.....	78	45	61	38
2 at 1.5 hours.....	35	29	33	27
3 - 2.....	43 = C	16 = K	28 = U ₁	11 = U ₂
T, from nomograph 1.....		35	17	56
Concentration, from nomograph 2, in units per ml.....			0.0194	0.064

Method 2. If method 2 alone is used, the only readings necessary are readings 1 and 4. Subtract reading 1 from reading 4 for each tube. Let this difference for the first tube be called *C*, the second tube *K*, and for the unknown tubes *U*. Subtract *K* from *C* and each of the *U* values from *C*. Then read the units of penicillin per ml. in the unknown tubes at the intersection with the diagonal line of a straight line from *C* - *K* on the left hand column to *C* - *U* in the right hand column of nomograph 3. If the known had a concentration other than 0.04 unit per ml., use the multiplication factor. Multiply results as read from the nomograph by the dilution factor, usually 10, and by any preceding dilutions to get the concentration in the original specimen.

Example, method 2:

READING	CONTROL, TUBE 1	KNOWN, TUBE 2	UNKNOWN	
			Tube 3	Tube 4
4 at 18 hours.....	242	81	143	55
1 at 0 hours.....	22	24	27	30
4 - 1.....	220 = C	57 = K	116 = U ₁	25 = U ₂
C - K or U.....		163	104	195
Units per ml. from nomograph 3.....			0.0195	0.057

RESULTS

Results are shown in Table 1. In comparing these with results by other methods keep in mind that these are results from many unknowns run against one control and one known, while most results reported for other methods are averages of several replicates of each unknown against a series of knowns and controls. Replicates may be used with these methods if increased accuracy is desired, but the results reported are sufficiently accurate for most purposes.

ASSAY FOR STREPTOMYCIN

Previously published methods for streptomycin assay⁵ have the same defects as the previously published penicillin assay methods.

The technic recommended for streptomycin assay is identical with that herein described for penicillin, with the following exceptions. One microgram of strep-

TABLE 1
SUMMARY OF RESULTS OF PENICILLIN ASSAY OBTAINED BY EACH OF TWO
NOMOGRAPHIC METHODS

ADDED	FOUND		AVERAGE
	Method 1	Method 2	
0.090	0.105	0.075	0.090
0.080	0.106	0.1055	0.106
	0.0795	0.096	0.088
	0.152	0.090	0.121
	0.0845	0.075	0.080
	0.0845	0.074	0.079
	0.0653	0.083	0.074
	0.0604	0.060	0.060
	0.051	0.074	0.063
Average.....	0.0854	0.0822	0.0839
0.070	0.0845	0.071	0.077
0.060	0.0865	0.066	0.0763
	0.082	0.062	0.072
	0.064	0.0474	0.056
	0.065	0.066	0.065
	0.0855	0.066	0.076
	0.072	0.065	0.068
Average.....	0.0758	0.0621	0.0689
0.050	0.040	0.0460	0.043
	0.0775	0.0514	0.064
Average.....	0.0587	0.0487	0.0535
0.040	0.048	0.0412	0.0446
	0.040	0.0391	0.0395
	0.0360	0.0420	0.0390
	0.0472	0.0391	0.0432
	0.040	0.0395	0.0398
	0.047	0.0454	0.0462
	0.0373	0.0406	0.0389
	0.0424	0.0396	0.0410
	0.0424	0.0406	0.0415
	0.0373	0.0390	0.0382
	0.0424	0.0411	0.0417
	0.040	0.0386	0.0393
	0.0505	0.0416	0.0460
Average.....	0.0423	0.0406	0.0415

TABLE 1—Continued

ADDED	FOUND		AVERAGE
	Method 1	Method 2	
0.0350	0.0343	0.0343	0.0343
0.030	0.0244 0.0330 0.0325	0.0265 0.0267 0.0324	0.0254 0.0299 0.0325
Average.....	0.030	0.0285	0.0293
0.0250	0.0244 0.0280	0.0218 0.0255	0.0231 0.0268
0.020	0.0160 0.0160 0.0178 0.0135 0.020 0.0170 0.0095 0.0164 0.0184 0.020 0.0166 0.0136 0.0220 0.0230 0.0180 0.0312 0.0282 0.0170 0.0240 0.0195 0.0173	0.0159 0.0139 0.0186 0.0186 0.0158 0.0330 0.0156 0.0192 0.0188 0.0180 0.0170 0.0170 0.0228 0.0128 0.0166 0.0189 0.0199 0.0216 0.0222 0.0201 0.0216	0.0159 0.0150 0.0182 0.0161 0.0179 0.0250 0.0126 0.0178 0.0186 0.0190 0.0168 0.0153 0.0224 0.0179 0.0173 0.0250 0.0240 0.0193 0.0231 0.0198 0.0195
Average.....	0.0188	0.0189	0.0188
0.010	0.0090 0.0085 0.0072 0.0095 0.0070 0.0130 0.0070 0.0025	0.0057 0.0073 0.0076 0.0079 0.0065 0.0084 0.0068 0.0119	0.0074 0.0079 0.0074 0.0087 0.0068 0.0107 0.0069 0.0072
Average.....	0.0080	0.0078	0.0079
0.0080	0.0060 0.0060 0.0090	0.0040 0.0026 0.0054	0.0050 0.0043 0.0072
Average.....	0.0070	0.0040	0.0055

tomycin equals one unit, so the terms units and micrograms are interchangeable and 1 mg. of streptomycin equals 1000 units. Two knowns are necessary instead of one known, with final concentrations of 1 unit and 3 units per ml. and for method 2, if read after six hours, a third known containing 10 units per ml. is desirable. These are prepared by adding 0.5 ml. of a 10 unit per ml. dilution to tube 2, 0.5 ml. of a 30 unit per ml. dilution to tube 3, and, if method 2 is to be read the next day, 0.5 ml. of a 100 unit per ml. concentration to tube 4. Use tubes 2 and 3 for the knowns in methods 1 and 2, if readings for method 2 are made between four and six hours as is recommended, and tube 4 for the known in method 2 if readings are made the next day. For method 2, use nomograph 4 instead of nomograph 3. The effective range is 0.2 to 20.0 units per ml. in the final culture corresponding to a range of 2 to 200 units or micrograms per ml. in the original material when 0.5 ml. is used. Sensitivity is increased by slowing of the growth rate or decreasing the inoculum, and lower concentrations may be determined by using 1 or 2.5 ml. of serum and correspondingly less broth culture and multiplying by 5 or 2 instead of 10.

Urine excreted during the first two hours after a dose of streptomycin should be diluted to a volume of 50 ml. per min. and 0.5 ml. used.

ASSAY OF TRIVALENT ORGANIC ARSENICALS

Neoarsphenamine has been shown to be an extremely effective antibacterial and antimalarial agent, both clinically when so administered as to maintain uniform blood concentrations²¹ and *in vitro*.²⁰ The other trivalent organic arsenicals are very effective *in vitro*²⁰ and are much used in the treatment of syphilis and other treponemal infections, and no clinically practical good method for determination of the active form of these drugs has previously been available.

The technic of determining these compounds is the same as that given for streptomycin, with these exceptions: For neoarsphenamine, use a known of 1.5 micrograms per ml. concentration, corresponding to the addition of 0.5 ml. of a *freshly prepared* 1:10,000 dilution of the contents of a 0.15 gm. ampule. Use a concentration of the other trivalent organic arsenicals, except mapharsen, of equivalent arsenic content.²⁰ Use a standard of half this strength, corresponding to 0.5 ml. of 1:10,000 dilution of a 75 mg. ampule of mapharsen or related arsenoxides. In calculation, use the multiplication factor from nomographs 2 and 4 times the known concentration, for the values of the unknowns. The range covered is from 0.2 to 3.0 micrograms of neoarsphenamine per ml. Because of this extreme sensitivity, blood serum may have to be diluted 1:2 to 1:10, when 0.5 ml. is used. Blood serum should be collected between one and eight hours after the last dose and the time elapsed since the last dose accurately recorded.

COMMENT

These methods are much simpler to use than the standard method for sulfonamide determination and many more determinations may be done in a day by one technician. However, because sulfonamides are not effective against large numbers of organisms this method is not suitable for sulfonamide determination.

With little or no modification in technic it should be satisfactory for other antibiotics which we have not tested, such as streptothricin.

It is interesting to note that with method 2 the differences in reading for the penicillin-containing cultures are proportional to the square root of the concentration, while with all other antibacterial agents tested, the difference is directly proportional to the concentration. This may be due to the disintegration of the penicillin with time at the 37° temperature.

A single serum level obviously does not indicate what the blood level of any of the compounds would be before or after the time at which the sample was taken. If, however, the time since the last dose was administered is known, a satisfactory clinical approximation will be obtained, if it is assumed that the concentration is uniform when the drug is given by continuous drip and changes by the factors indicated below if given by other routes.

DRUG	INTERMITTENT ADMINISTRATION	FACTOR	TIME
			hours
Penicillin.....	Intramuscular	2	0.5
Penicillin.....	Intravenous	4	0.5
Penicillin.....	Oral	2	1.0
Penicillin in beeswax.....	Intramuscular	2	4.0
Streptomycin.....	Intramuscular	2	2.0
Streptomycin.....	Intravenous	2	1.5
Neoarsphenamine.....	Intravenous	2	6.0

For example, if the blood level of penicillin one hour after an intramuscular injection is 0.4 unit per ml. it was 0.8 unit per ml. thirty minutes after the injection; it will be 0.2 unit one and one-half hours after injection, 0.1 unit at two hours, and 0.05 unit at two and one-half hours after the same injection. Penicillin absorption after oral administration is so variable in different individuals that quantitative blood determinations will be essential in following patients on such therapy. More accurate methods of predicting blood levels are given in reference 19.

SUMMARY

A simple and accurate method for the quantitative determination of penicillin, streptomycin, neoarsphenamine and other antibacterial agents is outlined, requiring only one control and one or two knowns for many unknowns.

Nomograms are published permitting rapid determination of results by either or both of two methods.

Results by method 1 are available within three hours.

Table 1 shows results for 69 knowns run as unknowns and determined by each of the nomographic methods of estimation.

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STUDIES OF UROBILINOGEN. VI.

FURTHER EXPERIENCE WITH THE SIMPLE QUANTITATIVE EHRLICH REACTION. CORRECTED CALIBRATION OF THE EVELYN COLORIMETER WITH A PONTACYL DYE MIXTURE IN TERMS OF UROBILINOGEN*

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In part V of the present series of papers, a simple method was described⁸ for the quantitative recording of the Ehrlich reaction, as carried out with urine and feces. This method may be employed with the Evelyn photo-electric colorimeter or with a comparator block with a series of standard dilutions of a suitable mixture of pontacyl carmine and pontacyl violet. The results with the two methods of colorimetry were shown to be in excellent agreement.

From the number of queries which have been received, it would appear, however, that the description of the method was inadequate in certain respects, and that as a result, some difficulty has been encountered in making use of it. Furthermore, we have subsequently noted a rather curious discrepancy with relation to the calibration of the Evelyn photo-electric colorimeter for this determination. The purposes of the present communication are: to present a corrected calibration; to clarify portions of the previous description which have appeared ambiguous to some; and to present certain additional data which one may gain from the simple quantitative Ehrlich reaction on a single, or a two hour, sample of urine, as compared with the quantitative determination of urobilinogen in a twenty-four hour sample.

A. CORRECTED CALIBRATION OF THE EVELYN PHOTO-ELECTRIC COLORIMETER

Pontacyl Dye Standard for Quantitative Determination of the Ehrlich Reaction in Terms of Urobilinogen

In paper IV,⁵ data were presented on the basis of which our Evelyn photo-electric colorimeter was calibrated for standard stercobilinogen and mesobilirubinogen aldehyde solutions. In paper V, Table 1,⁸ data were presented for comparisons of a series of dilutions of the pontacyl stock standard solution with urobilinogen aldehyde solutions of known concentration. This table is misleading in that it indicates that a series of Evelyn readings is equivalent, on the one hand, to the dilutions of the dye in the standard, and on the other, to the urobilinogen concentrations shown. As a matter of fact, these assumptions are incorrect. Actually, the urobilinogen aldehyde solutions (the urobilinogen concentration of which was determined by reference to Table 2 in paper IV), were

* Aided by a grant from the Medical Research Fund of the Graduate School, University of Minnesota. Received for publication, September 30, 1946.

found to be equivalent with the dilutions of the pontacyl dye, not by means of the Evelyn colorimeter, but by direct comparison in a Duboscq colorimeter. Unfortunately, this point was not made clear in paper V. A visual comparison was made purposely, since it was desired to use these dye solutions for direct visual colorimetry in the comparator block. The Evelyn readings in Table 1 of paper V are the readings obtained with the dye dilutions given in the table, not with the urobilinogen aldehyde solutions. This is undoubtedly the reason for the discrepancy which is noted between the data in Table 2, paper IV, and Table 1, paper V. This discrepancy in values is based simply upon the different spectral distribution curves of the pontacyl dye standard and the urobilinogen aldehyde solution, as compared in Fig. 1. It is seen that the absorption of the

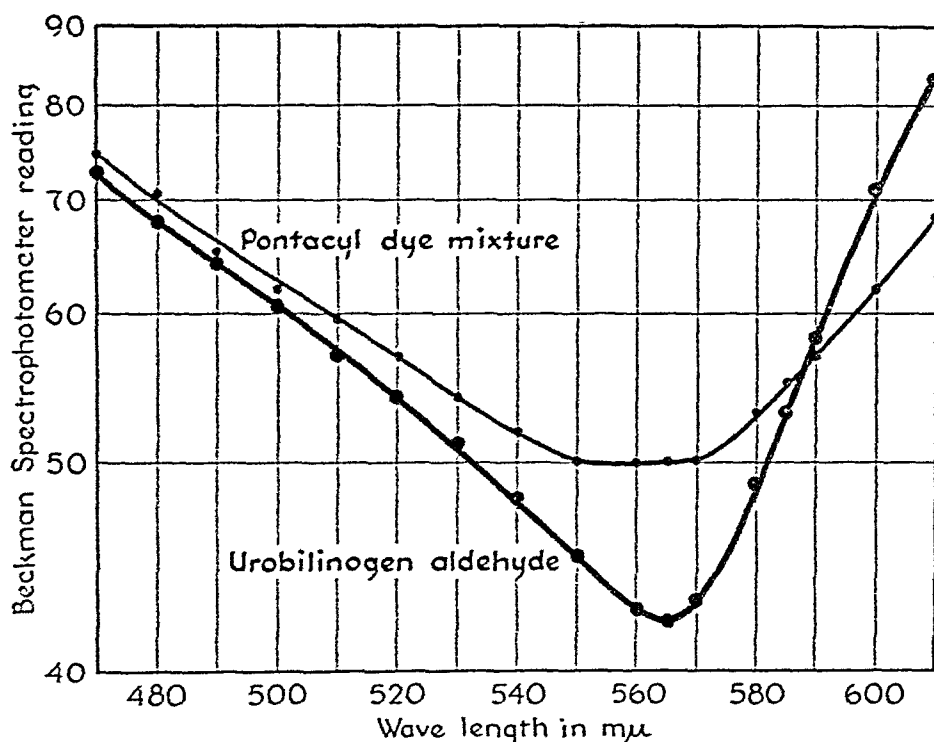


FIG. 1

former is relatively broader and more diffuse than that of the latter. If these two solutions are compared with an ordinary spectroscope, the difference is even more striking. Nevertheless, when viewed with the naked eye, or in the Duboscq colorimeter, the colors of the two solutions match perfectly. Thus, the difference in spectral distribution curves does not in any way detract from the value of the pontacyl dye standard as far as visual color comparison with the urobilinogen aldehyde solution is concerned. It does, however, require a correction to permit use of the dye standard for calibration of an Evelyn photo-electric colorimeter in terms of known concentrations of urobilinogen. Thus, if the Evelyn readings in Table 1, paper V, were used, rather than those in Table 2, paper IV, an error ranging from 5 to 20 per cent would be introduced, depending on the relative concentration of the urobilinogen aldehyde solution.

In the present study, the same stock standard pontacyl dye solution was used. This consists of a solution of 95 mg. of pontacyl violet 6R 150 per cent* and 5 mg. of pontacyl carmine 2B* dissolved in 1000 cc. of 0.5 per cent glacial acetic acid. An aliquot of this 10 mg. per cent stock standard diluted to 2.04 mg. per cent gives the same reading on the Evelyn colorimeter as that of a 0.60 mg. per cent urobilinogen (aldehyde) solution. The further dilutions of the dye and the urobilinogen concentrations, as given in Table 1, have the same absorption readings on the Evelyn photo-electric colorimeter using a filter with maximum transmission at 565 m μ .

The graph obtained with the above data may be superimposed with that obtained from the data given in paper IV, Table 2, for pure stercobilinogen and mesobilirubinogen aldehyde solution. It is thus possible to calibrate any other colorimeter of this type by means of the above pontacyl concentrations.

TABLE 1

CALIBRATION OF AN EVELYN COLORIMETER FOR DILUTIONS OF THE STOCK PONTACYL DYE MIXTURE AS COMPARED WITH UROBILINOGEN ALDEHYDE SOLUTIONS OF KNOWN CONCENTRATION

PONTACYL DYE MIXTURE IN MG. PER 100 CC.	UROBILINOGEN IN MG. PER 100 CC.	READINGS ON THE EVELYN COLORIMETER
2.040	0.6	10 ²
1.700	0.5	15 ²
1.360	0.4	22 ²
1.020	0.3	32 ²
0.850	0.25	39 ¹
0.680	0.20	47 ¹
0.510	0.15	57
0.340	0.10	68 ²
0.170	0.05	83
0.085	0.025	91

It should be noted that these are not identical with the values given in paper V,⁸ for use in the comparator block. The reason for this undoubtedly lies in the difference between the spectral and visual color characteristics of the dye, as already mentioned. In paper V, the actual concentrations of the dye mixture used in the comparator block method were not given, it being stated merely that a series of unspecified dilutions of a 1 to 6 dilution of the stock standard was employed. Since some question has arisen about these dilutions, the exact concentrations of the dye mixture which are recommended for use in the comparator block are given in Table 2.

B. FACTORS CONCERNING THE EHRLICH REACTION ITSELF

It has long been known that the color of the Ehrlich aldehyde reaction as developed in the urine, deepens gradually as time elapses from the onset of the

* This is the designation used by E. I. duPont deNemours and Company, Inc., Wilmington, Del.

reaction. Our previous experience with this deepening had indicated that it varied markedly under different conditions and with different urine samples. Hence, it was recommended in paper V that the reaction be carried out as follows: "The sample is measured and after cooling to room temperature, 2.5 cc. are mixed with 2.5 cc. of Ehrlich's reagent in an Evelyn tube. Five cc. of a saturated solution of sodium acetate are then added and thoroughly mixed." Although this last sentence may have been somewhat ambiguous, it was intended to indicate that the sodium acetate is added immediately after a thorough mixing of the urine with the Ehrlich reagent. More recently, Kelly, Lewis and Davidson³ have studied the deepening of the color reaction with time and have found that it does not appear to be related to urobilinogen. They believe, since it is obviously impossible to add the sodium acetate without the passage of any time whatever, that a fifteen second interval is optimal. Our experience indicates that timing of an interval is unnecessary and that the procedure as first described

TABLE 2
CONCENTRATIONS OF PONTACYL DYE MIXTURE RECOMMENDED FOR USE IN COMPARATOR BLOCK, TOGETHER WITH EQUIVALENT CONCENTRATIONS OF UROBILINOGEN

PONTACYL DYE MIXTURE IN MG. PER 100 CC.	UROBILINOGEN IN MG. PER 100 CC.
1.667	.60
1.389	.50
1.111	.40
.833	.30
.699	.25
.555	.20
.416	.15
.277	.10
.138	.05
.069	.025

is quite satisfactory, assuming that the term "sodium acetate are then added" is interpreted to mean without delay. Thus, one should not, in carrying out determinations on a number of urine samples, add the Ehrlich's reagent to the entire series before adding the sodium acetate to any. In other words, the latter should be added to each tube immediately after the urine has been shaken with Ehrlich's reagent. In this connection, it should be emphasized that the sodium acetate should be pure, and that the solution should be saturated. Impurities have been shown to affect the color of the aldehyde compound,⁵ and if the solution is not saturated, the amount added may be insufficient to convert all of the hydrochloric acid in the Ehrlich's reagent to acetic acid. This is of particular importance insofar as the blank solution is concerned, since if the hydrochloric acid is not fully changed to acetic acid, a partial Ehrlich reaction will ensue and the resultant value in Ehrlich units will be too low. Some question has arisen about the speed of fading of the color after the sodium acetate has been added. We have studied this repeatedly and find no significant fading within five minutes, if pure sodium acetate is used.

C. FURTHER COMPARATIVE DATA RELATIVE TO UROBILINOGEN IN TWENTY-FOUR HOUR SAMPLES OF URINE AND FECES

Single Sample Quantitative Ehrlich Reaction

Since a considerable variation exists in urobilinogen excretion in urine at different times during the twenty-four hours,⁸ we have compiled additional data comparing the results obtained in Ehrlich units in 2 to 4 P. M. specimens with those obtained in milligrams of urobilinogen for the same day. These data, as given

TABLE 3

FURTHER DATA ON THE 24 HOUR UROBILINOGEN AND THE EHRLICH UNITS PER TWO HOUR SAMPLE DURING THE SAME 24 HOURS

CASE NUMBER	EHRLICH UNITS IN 2 TO 4 P. M. SPECIMEN	MG. OF UROBILINOGEN PER 24 HOURS
1	4.5	25.6
2	3.7	24.5
3	1.9	14.9
4	20.3	36.2
5	20.3	65.0
6	18.4	44.0
7	22.4	78.0
8	50.6	72.0
9	1.3	4.2
10	1.1	4.7
11	1.0	3.2
12	0.5	0.3
13	0.4	1.5
14	0.8	0.2
15	0.9	0.5
16	0.7	0.3
17	0.4	0.4
18	0.4	1.5
19	5.1	3.4
20	1.4	0.6
21	0.1	4.2
22	0.1	5.7
23	1.0	0.9
24	1.0	10.4
25	5.6	7.8

in Table 3, again bear out the fact that a high urobilinogen excretion over the twenty-four hour period is generally associated with an increase in the 2 to 4 P. M. sample.

There are some exceptions, however, of which case number 19 is an example. In this instance, 5.1 Ehrlich units were found in the two hour sample, while the twenty-four hour urobilinogen was just at the upper limit of normal. The question arises as to whether most of the urobilinogen was excreted between 2 and 4 P. M. While this occurrence may at least partly account for the difference,

other possibilities must be considered. For one thing, there may have been a loss during the petroleum ether extraction. A discussion of this point was presented in the preceding papers.^{5, 8} Another possibility is that during the twenty-four hour urine collection, the urobilinogen may have been oxidized to substances that could not be reduced back to urobilinogen. This has been discussed previously.^{6a, b}

A discrepancy of the opposite type is noted in cases 22 and 24, in which there was an increase in twenty-four hour urobilinogen excretion with a low 2 to 4 P. M. excretion. In case 24 particularly, this discrepancy was of considerable practical significance, in that while this individual quite clearly had other evidences of chronic active hepatitis, the value of the Ehrlich units in the 2 to 4 P. M. urine sample was within normal limits on each of three successive days. We can merely speculate that in instances such as this, the main urobilinogen excretion occurs at some time other than during the middle of the afternoon. Because of other experiences of the same type, we have adopted the policy of determining the twenty-four hour urine urobilinogen in instances in which evidence of liver injury is being sought, and in which two 2 to 4 P. M. samples have been found to be normal insofar as the Ehrlich reaction is concerned. The twenty-four hour quantitative determination, involving petroleum ether extraction, is also done in instances where there is considerable albuminuria causing interference by turbidity. Difficulty has also been encountered in applying the simple quantitative Ehrlich reaction to certain urines rich in bilirubin. In the latter instance, particularly if the urobilinogen content is very low, the color may be masked by the intense yellowness of the urine, or the green Ehrlich reaction¹ may interfere. In such instances, the urines should likewise be reduced with ferrous hydroxide and extracted by petroleum ether.⁵

In many cases in which complete exclusion of bile from the intestinal tract undoubtedly existed, the number of Ehrlich units in the two hour sample has been noted to be considerably higher than the number of milligrams of urobilinogen found in a twenty-four hour sample from a successive period of time. Whether this discrepancy is due to difference between the blank solution and that in which the aldehyde is added first, and is possibly occasioned by oxidation products of bilirubin, or whether it is due to substances other than urobilinogen which react with Ehrlich's reagent, is not clear. In any event, it is evident that values of 2 to 3 Ehrlich units per two hour sample may at times be observed in persons in whom the twenty-four hour urinary urobilinogen is less than 0.5, and the *per diem* fecal urobilinogen less than 5.0 mg.; in other words, in persons in whom there is complete exclusion of bile from the intestine. The occurrence of these relatively high Ehrlich values under such circumstances, must be thoroughly appreciated in using the simple method.

In accordance with the purpose for which it was initially devised, the simple quantitative Ehrlich reaction has proved of much value as a screening method, and for frequent serial usage in following cases of liver disease. If the above limitations are recognized, the method will often give valuable information with a minimum expense of time and material. A case in point, which has been

studied recently on the medical service of the University of Minnesota Hospital, may be cited briefly as follows.

E. R., a male, aged 25, had been exposed to two patients with epidemic hepatitis about four weeks prior to onset of his symptoms on August 2, 1946. He was first seen on August 4, complaining of anorexia, weakness and mild pain in the right upper quadrant of the abdomen. He had no jaundice and his temperature was normal. The only positive physical finding was definite tenderness over the liver. The liver, however, was not palpable. The laboratory findings were as follows: leukocytes, 8850 with 65 per cent neutrophils, 28 per cent lymphocytes, 5 per cent monocytes, 2 per cent eosinophils; the routine urinalysis was negative; bilirubin could not be demonstrated on this or subsequent days, either by the barium strip (Harrison) test² or the methylene blue test.¹ On August 4, however, the morning urine sample contained 5.6 Ehrlich units per 100 cc. and the same concentration was observed in a single afternoon sample of that date. The twenty-four hour urine urobilinogen value from August 4 to August 5, was 7.8 mg. The prompt reacting (one minute) serum bilirubin was 0.25 mg. per 100 cc., a value slightly above the upper limit of normal in our experience.⁹ The total bilirubin was likewise slightly elevated, 1.7 mg. per 100 cc. The serum alkaline phosphatase was normal, 2.9 Bodansky units. The total cholesterol was 192 mg. per 100 cc., with 74 per cent esters. The cephalin cholesterol flocculation test was negative, as was the thymol turbidity test. The urinary coproporphyrin was well within normal limits, 39 gamma in twenty-four hours. All of these results were obtained on August 4. The bromsulfalein test on August 8 revealed but 2.5 per cent retention forty-five minutes after injection of 5 mg. of the dye per kilogram of body weight. The urine Ehrlich reaction on August 5 was 1.5 units and on August 6, 0.4 units.

From this report it is apparent that the test was significantly positive only at the onset of the disease, and that it was the only laboratory procedure, with the possible exception of the slightly elevated serum bilirubin, which aided in confirming the diagnosis of hepatitis. It is true, however, that the bromsulfalein test was not done at the outset. The patient recovered quickly, and after one week in bed, felt entirely well. This is believed to represent an instance of an abortive attack of hepatitis without jaundice.

The Ehrlich determination on random samples of feces has proved of considerable diagnostic value, especially in establishing quickly the presence of excessive hemolysis. Values above 400 Ehrlich units per 100 gm. are believed to indicate an increased rate of blood destruction, with the possible exception of occasional cases in which diarrhea interferes with absorption of urobilinogen. This possibility is mentioned, although we have not encountered it. Values between 250 and 400 units per 100 gm. are regarded as borderline and require either serial determinations or a four day collection with determination of the excretion of urobilinogen *per diem*. The latter method is undoubtedly superior insofar as an exact study of hemoglobin metabolism is concerned. On the other hand, a single quantitative Ehrlich determination on a random sample of feces may be decisive in the diagnosis of hemolytic anemia, and the result may be so definite that it is obviously unnecessary from the clinical standpoint to determine the daily excretion of urobilinogen over a four day period. The following example may be given.

M. M., a male, aged 64, on April 17, 1944 complained of weakness and anorexia of four months' duration and of jaundice and pallor of four weeks' duration. He had splenomegaly and moderate enlargement of the liver. Laboratory findings included hemoglobin, 4.0

gm. per 100 cc.; erythrocytes, 800,000 per cu. mm.; mean cell diameter 9.0 microns; leukocytes, 210,000, with 93 per cent lymphocytes, many of which were immature; total serum bilirubin, 3.7 mg. per 100 cc.; reticulocytes 33 per cent. The patient was so ill that a satisfactory collection of feces for quantitative determination of urobilinogen was not possible. An Ehrlich determination on a small amount of feces obtained by rectal examination revealed 1152 Ehrlich units per 100 gm. This confirmed the diagnosis of severe macrocytic hemolytic anemia with lymphatic leukemia.

In Table 4, some additional data are given to that presented in paper V, relating to comparison of values for Ehrlich units per 100 gm. of feces in a random sample, and for urobilinogen in milligrams per day at the same period.

The above data are representative of our experience and indicate that for routine clinical work, the Ehrlich determination on a random sample will often give all the information needed. A single determination on one day is insufficient to prove the presence of complete biliary obstruction, such as is characteristic of cancer of the biliary tract.⁷ If less than 5 units per 100 gm. are observed on each of four random samples during as many days, this is believed equivalent

TABLE 4

EHRlich UNITS PER 100 GM. OF A RANDOM SAMPLE OF FECES COMPARED WITH MG. OF UROBILINOGEN PER DAY FOR A FOUR-DAY PERIOD

CASE NUMBER	DIAGNOSIS	EHRlich UNITS PER 100 GM. FECES	MG. URO- BILINOGEN PER DAY
1	Familial hemolytic anemia	1280	804
2	Carcinoma of head of pancreas	2.3	2.2
3	Postoperative common bile duct stricture	13.0	9.2
4	Cirrhosis of the liver with jaundice	107.0	51.0
5	Familial hemolytic anemia	1720.0	1086.0
6	Carcinoma of the ampulla of Vater.	4.8	1.2

to the finding of less than 5 mg. of urobilinogen per day for an entire four day collection.⁷ Values between 5 and 10 Ehrlich units per 100 gm. must be regarded as borderline, at least for the present, while values over 10 units are believed to indicate that some bile is gaining access to the intestine.

SUMMARY AND CONCLUSIONS

A corrected calibration is given of the Evelyn photo-electric colorimeter with a pontacyl dye mixture, in terms of urobilinogen.

The finding of occasional discrepancies between the twenty-four hour urine urobilinogen and the 2 to 4 P.M. quantitative Ehrlich reaction, is discussed. Because of instances in which the value in the latter test is normal and that in the former test distinctly elevated, it is recommended that, if liver disease or injury is suspected, and two single samples or a sample collected over a short period have given normal results, a twenty-four hour urobilinogen be determined.

In instances where the urine contains much albumin, or large amounts of bilirubin, especially if with the latter a green Ehrlich reaction is encountered, it is re-

commended that, following ferrous hydroxide reduction and petroleum ether extraction, the urobilinogen be determined in a twenty-four hour specimen.

Further study of the Ehrlich determination on random samples of feces has confirmed the frequent usefulness of this method especially in quickly determining excessive hemolysis. Values above 400 Ehrlich units per 100 gm. are believed indicative, with rare exceptions, of an increased excretion of urobilinogen in the feces. Values less than 5 units per 100 gm., if encountered in four daily random samples, are believed equivalent to a finding of less than 5 mg. of urobilinogen per day for an entire four day collection of feces, insofar as determining complete exclusion of bile from the intestine is concerned.

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THE EFFECT OF LIPIDS ON KAHN ANTIGEN

I. REDUCTION OF SENSITIVITY BY ADDITION OF LECITHIN*

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In the standardization of Kahn antigen, it is not uncommon to find that the original antigen prepared from beef heart powder is oversensitive or undersensitive. Usually these variations in sensitivity are of a moderate degree and it is possible to correct the antigen to the sensitivity level of standard Kahn antigen by several technical steps.¹ In some instances, however, an antigen is so undersensitive or so oversensitive that the described methods of correction are not applicable. In the preparation of Kahn sensitized antigen for the presumptive test, a lipid mixture (sensitizing reagent) is added to standard Kahn antigen to increase its sensitivity to the level of sensitized antigen. The basis for the standardization of either standard or sensitized antigen is the adjustment to proper sensitivity by varying the lipid concentration of the antigen. This fact led us to investigate the effects of adding certain lipids, such as lecithin or cephalin, to Kahn antigen. It was believed that individual lipids in antigen standardization would be more desirable than lipid mixtures. In this article, data will be presented indicating that lecithin added to Kahn antigen reduces its sensitivity; also, that this property of lecithin can be utilized in adjusting highly oversensitive antigens to standard requirements, even when they were originally beyond the range of correction by the usual methods.

MATERIALS AND METHODS

Antigens. Seven uncorrected Kahn antigens were selected: two oversensitive antigens, O₁ and O₂, which could be corrected by routine methods; two oversensitive antigens, O₃ and O₄, which could not thus be corrected; two undersensitive antigens, U₁ and U₂, which could not be corrected by routine methods; and one undersensitive antigen, U₃, which could be thus corrected.

Lecithin. Seven different solutions of lecithin in absolute ethyl alcohol, A to H, were prepared and purified according to Pangborn₂ and were used after the addition of 0.6 per cent cholesterol. An eighth lecithin solution, J, was prepared by extracting a 5 per cent suspension of nonpurified commercial egg lecithin with 0.6 per cent cholesterolized absolute alcohol for one hour in a water bath at 56 C. This solution was cooled and filtered. It was found to contain 8.48 per cent phosphorus. The concentration and source of the lecithin solutions were as follows:

* Aided by a grant from the Difco Laboratories, Inc., Detroit, Michigan. Received for publication, September 28, 1946.

SOLUTION	PERCENTAGE OF LECITHIN	SOURCE*
A	1.43	Beef Heart CM
B	0.89	Beef Heart CM
C	2.12	Beef Heart CM
D	2.91	Beef Heart MP
E	1.45	Beef Heart MP
F	2.85	Soy bean CM
G	3.36	Soy bean MP
H	4.98	Soy bean CM
J	—	Egg-Difco

* Extracts marked CM were prepared by Drs. Martin Chanin and Stanley Marcus in this laboratory; those marked MP, by Dr. Mary C. Pangborn in the New York State Health Department Laboratories; egg lecithin for solution J was kindly supplied in generous amounts by the Difco Laboratories, Inc., Detroit, Michigan; solution E was a 50 per cent dilution of lecithin D with 0.6 per cent cholesterolized absolute alcohol; solution B, in contrast to the other six purified lecithin solutions which were colorless, had a pale amber color.

Choline hydrochloride. A solution of choline was prepared by adding 30 gm. of choline hydrochloride to 100 ml. of 95 per cent alcohol. Cholesterol was not added to this solution since it was found to precipitate at room temperature.

*Method of Antigen Titration.*¹ Four suspensions of each antigen were prepared by mixing 1 ml. quantities of antigen with 1.1, 1.3, 1.5 and 1.7 ml. of 0.9 per cent sodium chloride solution. A control was set up at the same time using standard Kahn antigen at the indicated titer. In titrating each antigen suspension, the procedure of the three tube Kahn test was employed, except that 0.9 per cent salt solution was used instead of serum. After the usual three minute shaking period, 1 ml. of 0.9 per cent salt solution was added to each tube containing 0.05 ml. of antigen suspension, and 0.5 ml. salt solution to each of the remaining tubes. The racks were shaken by hand for a few seconds to insure thorough mixing and the degrees of turbidity or clarity of the three tube tests were read.

The titer was considered to be the minimum amount of salt solution added to 1 ml. of antigen, producing a suspension of aggregates which undergoes visually complete dispersion upon the addition of further salt solution, resulting in an homogeneous opalescent liquid. Some of the antigen suspensions in the titration range contained aggregates which were not dispersible; others contained aggregates which were so completely dispersed as to lead to water clarity. The notations of the titration readings, ranging from heavy nondispersible aggregates to water clarity, are listed below. The abbreviated forms are designated when abbreviations are used.

Heavy aggregates—Heavy agg.
 Cloudy, aggregates—Cloudy, agg.
 Cloudy, fuzzy
 Cloudy
 Slightly cloudy—Sl. cloudy
 Trace cloudy—Tr. cloudy
 Opalescent

Trace clear—Tr. clear
 Slightly clear—Sl. clear
 Slightly too clear—Sl. too clear
 Too clear
 Watery clear

The following outline presents the titration pictures of an oversensitive antigen, of an undersensitive antigen, and of standard Kahn antigen.

AMOUNTS IN ML. OF ANTIGEN AND SALT SOLUTION	OVERSENSITIVE ANTIGEN	UNDERSENSITIVE ANTIGEN	STANDARD KAHN ANTIGEN
1 + 1.1	Heavy agg.	Tr. clear	Tr. cloudy
1 + 1.3	Heavy agg.	Watery clear	Opalescent
1 + 1.5	Cloudy, agg.	Watery clear	Sl. clear
1 + 1.7	Cloudy	Watery clear	Too clear

Thus, an antigen which gives titration readings that range from "heavy aggregates" to "cloudy" is oversensitive, while an antigen which gives titration readings ranging from "trace clear" to "watery clear" is undersensitive. The desirable titration picture is the one in which the antigen suspensions made with increasing volumes of salt solution, that is, 1.1, 1.3, 1.5 and 1.7 ml., show a gradual increase in clarity. The titration end point or titer is the tube which shows opalescence.

After the titer of an antigen was determined, the next step was to establish whether the sensitivity and specificity of the antigen were comparable to those of Kahn standard antigen. This was accomplished by performing Kahn tests with the new antigen and with standard antigen simultaneously, employing two negative and eight weakly positive serums. If the sensitivity of the newly prepared antigen differed from that of standard antigen, further steps were used to correct it to standard requirements.¹

Methods of Correction. After the preliminary titrations, 10 ml. samples of each antigen were diluted 1 per cent, 5 per cent and 10 per cent, respectively, with a given lecithin solution and were retitrated. If a satisfactory titer was not quite reached, other dilutions with lecithin were employed, such as 3 per cent or perhaps more than 10 per cent, according to the indications of the preceding titration. For control purposes, titrations were performed with the antigens after similar dilutions with cholesterolized alcohol.

After satisfactory titrations of the antigens had been obtained, the 10 ml. samples were tested with weakly positive syphilitic serums to determine their sensitivities. For, while a satisfactory titer is a prerequisite for an antigen, it is no definite indication of correct sensitivity. If it was not closely comparable to standard sensitivity, further corrections were made by the usual procedure of dilution of the antigen with cholesterolized alcohol and/or by small adjustments in the amount of salt solution used in the titer. Finally, a 50 ml. sample of each antigen was prepared according to the method used in making the 10 ml. test samples and rechecked with standard Kahn antigen.

EXPERIMENTAL

1. *The effect of lecithin on the titration picture of oversensitive antigens.* Four oversensitive antigens, O₁, O₂, O₃ and O₄, were each diluted 1 per cent, 5 per cent and 10 per cent with lecithin solution C, and the diluted antigens titrated with 0.9 per cent salt solution. Control titrations were performed with the antigens after similar dilutions with cholesterolized alcohol. The results of this experiment are given in Table 1.

It can be seen from the table, that none of the original undiluted antigens or portions of these antigens diluted with cholesterolized alcohol gave an acceptable titration picture and, hence, all were unfit for use with serum. On the other hand, antigen O₁ diluted with 5 per cent of the lecithin solution and antigen O₂ diluted with 1 per cent, gave typical titration pictures of usable antigens. It is also evident from the table that the 5 per cent lecithin dilutions of antigens O₃ and O₄ reduced the turbidity of the titration mixtures beyond the titer, and that a dilution of the antigens with lecithin between 1 per cent and 5 per cent would give an acceptable titer. Determinations of the optimal amount of lecithin to be added to an oversensitive antigen will be presented below.

2. *The effect of lecithin on the titration picture of undersensitive antigens.* An experiment was carried out with three undersensitive antigens, U₁, U₂ and U₃, in exactly the same manner as described for oversensitive antigens in experiment 1. The results are presented in Table 2.

It is shown in Table 2 that dilutions with lecithin or cholesterolized alcohol did not markedly alter the titration pictures of antigen U₁ and U₂. Antigen U₃ was a special type of undersensitive antigen in that a titer could be obtained either in the undiluted state or after dilution with cholesterolized alcohol. In both instances, however, a "flat" titration picture was obtained since the differences in the turbidity of suspensions made with 1.1, 1.3, 1.5 and 1.7 ml. of salt solution were very slight and did not show the desired progressively increasing clarity of a "sloping" titration picture; experience having shown that the latter picture is the desirable one. It can be seen from the table that the dilutions of antigen U₃ with lecithin changed the titration picture from a "flat" to a "sloping" titer.

3. *The effect of choline hydrochloride on the titration picture of antigen.* An experiment was undertaken to determine whether or not choline, a hydrolytic product of lecithin, too, would alter the sensitivity of oversensitive and undersensitive antigens and, if so, to what extent. Accordingly, oversensitive antigens, O₁, O₂ and O₃, and undersensitive antigens, U₁, U₂ and U₃, were diluted 1 per cent, 5 per cent and 10 per cent with a 30 per cent solution of choline hydrochloride in absolute alcohol. The results are summarized in Table 3.

It is seen in Table 3 that the titration pictures of oversensitive antigens diluted with choline show a slight reduction in turbidity which does not approach the decrease obtained by the use of dilutions with lecithin. In addition, the table shows that dilutions of undersensitive antigens with choline had no apparent effect on their titration pictures.

4. *The effect of various lecithin preparations on the titration picture of oversensitive antigens.* The original observations on the ability of lecithin to decrease the

TABLE 1
ILLUSTRATIONS ON THE EFFECT OF BEEF HEART LECITHIN ON THE TITRATION PICTURE OF OVERSENSITIVE ANTIGENS

AMOUNTS IN ML. OF ANTIGEN AND SALT SOLUTION	TITRATION READINGS OF UNCORRECTED ANTIGEN	TITRATION READINGS OF ANTIGENS DILUTED WITH VARIOUS PERCENT- AGES OF A 2.12 PER CENT SOLUTION OF BEEF HEART LECITHIN				TITRATION READINGS OF ANTIGENS DILUTED WITH VARIOUS PERCENT- AGES OF CHOLESTEROLIZED ALCOHOL (CONTROLS)			
		1 per cent	5 per cent	10 per cent		1 per cent	5 per cent	10 per cent	
		Antigen O ₁							
1 + 1.1	Heavy agg.	Cloudy	Cloudy	Tr. cloudy	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.	
1 + 1.3	Cloudy	Sl. cloudy	Opalescent	Too clear	Cloudy	Cloudy, agg.	Cloudy, agg.	Cloudy, agg.	
1 + 1.5	Sl. cloudy	Tr. cloudy	Too clear	Watery clear	Sl. cloudy	Cloudy	Cloudy	Cloudy	
1 + 1.7	Sl. clear	Tr. cloudy	Watery clear	Water clear	Opalescent	Tr. cloudy	Tr. cloudy	Tr. cloudy	
Antigen O ₂									
1 + 1.1	Cloudy, agg.	Tr. cloudy	Sl. clear	Too clear	Cloudy, agg.	Sl. cloudy	Tr. cloudy	Tr. cloudy	
1 + 1.3	Cloudy, agg.	Opalescent	Watery clear	Watery clear	Cloudy, agg.	Cloudy, agg.	Heavy agg.	Heavy agg.	
1 + 1.5	Opalescent	Too clear	Watery clear	Watery clear	Opalescent	Opalescent	Sl. cloudy	Sl. cloudy	
1 + 1.7	Too clear	Watery clear	Watery clear	Watery clear	Too clear	Too clear	Too clear	Sl. clear	
Antigen O ₃									
1 + 1.1	Tr. cloudy	Tr. cloudy	Sl. too clear	Too clear	Tr. cloudy	Tr. cloudy	Opalescent	Opalescent	
1 + 1.3	Sl. cloudy	Tr. cloudy	Watery clear	Watery clear	Tr. cloudy	Tr. cloudy	Sharp	Sharp	
1 + 1.5	Cloudy, fuzzy	Opalescent	Watery clear	Watery clear	Cloudy	Sl. cloudy	Tr. cloudy	Tr. cloudy	
1 + 1.7	Cloudy	Sl. clear	Watery clear	Water clear	Cloudy	Sl. cloudy	Tr. cloudy	Tr. cloudy	
Antigen O ₄									
1 + 1.1	Heavy agg.	Cloudy, agg.	Tr. cloudy	Too clear	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.	
1 + 1.3	Heavy agg.	Heavy agg.	Watery clear	Watery clear	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.	
1 + 1.5	Heavy agg.	Opalescent	Watery clear	Watery clear	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.	
1 + 1.7	Heavy agg.	Too clear	Watery clear	Watery clear	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.	

TABLE 2
EFFECT OF BEEF HEART LECITHIN ON THE TITRATION PICTURE OF UNDERSENSITIVE ANTIGENS

AMOUNTS IN ML. OF ANTIGEN AND SALT SOLUTION	TITRATION READINGS OF UNCORRECTED ANTIGEN	TITRATION READINGS OF ANTIGENS DILUTED WITH VARIOUS PERCENT- AGES OF A 2.12 PER CENT SOLUTION OF BEEF HEART LECITHIN				TITRATION READINGS OF ANTIGENS DILUTED WITH VARIOUS PERCENT- AGES OF CHOLESTEROLIZED ALCOHOL (CONTROLS)			
		1 per cent				1 per cent			
		5 per cent				5 per cent			
Antigen U ₁									
1 + 1.1	Sl. too clear	Too clear	Too clear	Too clear	Sl. too clear	Sl. too clear	Sl. too clear	Sl. clear	
1 + 1.3	Watery clear	Watery clear	Watery clear	Watery clear	Watery clear	Watery clear	Too clear	Sl. too clear	
1 + 1.5	Watery clear	Watery clear	Watery clear	Watery clear	Watery clear	Watery clear	Watery clear	Too clear	
1 + 1.7	Watery clear	Watery clear	Watery clear	Watery clear	Watery clear	Watery clear	Watery clear	Watery clear	
Antigen U ₂									
1 + 1.1	Tr. clear	Tr. clear	Tr. clear	Sl. too clear	Tr. clear	Opalescent	Opalescent	Opalescent	
1 + 1.3	Sl. too clear	Too clear	Too clear	Too clear	Sl. too clear	Too clear	Too clear	Sl. too clear	
1 + 1.5	Watery clear	Watery clear	Watery clear	Watery clear	Watery clear	Too clear	Too clear	Too clear	
1 + 1.7	Watery clear	Watery clear	Watery clear	Watery clear	Watery clear	Watery clear	Watery clear	Watery clear	
Antigen U ₃									
1 + 1.1	Tr. cloudy*	Tr. cloudy†	Opalescent†	Opalescent†	Tr. cloudy	Sl. cloudy	Sl. cloudy	Sl. cloudy	
1 + 1.3	Opalescent	Opalescent	Tr. clear	Sl. too clear	Opalescent	Opalescent	Opalescent	Opalescent	
1 + 1.5	Opalescent	Sl. clear	Sl. too clear	Too clear	Opalescent	Opalescent	Opalescent	Opalescent	
1 + 1.7	Sl. clear	Sl. too clear	Too clear	Watery clear	Sl. clear	Sl. clear	Sl. clear	Opalescent	

* "Flat" titration.

† "Sloping" titration (see text).

TABLE 3

EFFECT OF CHOLINE HYDROCHLORIDE ON THE TITRATION PICTURE OF OVERSENSITIVE AND UNDERSENSITIVE ANTIGENS

AMOUNTS IN ML. OF ANTIGEN AND SALT SOLUTION	TITRATION READINGS OF UNCORRECTED ANTIGEN	TITRATION READINGS OF ANTIGENS DILUTED WITH VARIOUS PERCENTAGES OF 30 PER CENT SOLUTION OF CHOLINE HYDROCHLORIDE*		
		1 per cent	5 per cent	10 per cent
Antigen O ₁				
1 ÷ 1.1	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.
1 ÷ 1.3	Cloudy	Cloudy	Sl. cloudy	Opalescent
1 ÷ 1.5	Sl. cloudy	Tr. cloudy	Tr. cloudy	Sl. clear
1 ÷ 1.7	Sl. clear	Sl. clear	Sl. clear	Sl. too clear
Antigen O ₂				
1 ÷ 1.1	Cloudy, agg.	Cloudy	Sl. cloudy	Sl. cloudy
1 ÷ 1.3	Cloudy, agg.	Heavy agg.	Sl. cloudy	Tr. cloudy
1 ÷ 1.5	Opalescent	Cloudy	Too clear	Watery clear
1 ÷ 1.7	Too clear	Too clear	Watery clear	Watery clear
Antigen O ₃				
1 ÷ 1.1	Tr. cloudy	Opalescent	Tr. cloudy	Tr. cloudy
1 ÷ 1.3	Sl. cloudy	Tr. cloudy	Tr. cloudy	Tr. cloudy
1 ÷ 1.5	Cloudy, fuzzy	Tr. cloudy	Tr. cloudy	Tr. cloudy
1 ÷ 1.7	Cloudy	Tr. cloudy	Tr. cloudy	Tr. cloudy
Antigen U ₁				
1 ÷ 1.1	Sl. too clear	Sl. too clear	Too clear	Too clear
1 ÷ 1.3	Watery clear	Too clear	Watery clear	Watery clear
1 ÷ 1.5	Watery clear	Watery clear	Watery clear	Watery clear
1 ÷ 1.7	Watery clear	Watery clear	Watery clear	Watery clear
Antigen U ₂				
1 ÷ 1.1	Tr. clear	Sl. too clear	Sl. clear	Sl. too clear
1 ÷ 1.3	Sl. too clear	Too clear	Sl. too clear	Sl. too clear
1 ÷ 1.5	Watery clear	Too clear	Too clear	Watery clear
1 ÷ 1.7	Watery clear	Watery clear	Watery clear	Watery clear
Antigen U ₃				
1 ÷ 1.1	Tr. cloudy	Tr. cloudy	Tr. cloudy	Tr. cloudy
1 ÷ 1.3	Opalescent	Tr. cloudy	Opalescent	Opalescent
1 ÷ 1.5	Opalescent	Opalescent	Opalescent	Sl. clear
1 ÷ 1.7	Sl. clear	Too clear	Sl. clear	Sl. clear

* The control titration pictures of antigens diluted with cholesterolized alcohol are in Tables 1 and 2.

sensitivity of oversensitive antigen were made with lecithin solution B. This was an amber colored solution containing 0.89 per cent lecithin and was prepared

from beef heart muscle. During the course of this work other preparations of beef heart lecithin were made available and, in addition, two samples of lecithin from soy bean were obtained. A study was undertaken to determine the effect of the various lecithin solutions on oversensitive antigens, with special consideration given to the source of the lecithin. The results of one experiment are presented in Table 4.

It can be seen in Table 4 that the greatest effect on the titration picture occurred when dilutions were made with lecithin H, which was a soy bean extract containing 4.98 per cent lecithin. Lecithin G, another soy bean preparation containing 3.36 per cent lecithin, was the next most effective solution. The next four lecithin solutions, prepared from beef heart, showed progressively decreasing effects on the titration picture as the concentration of lecithin decreased. These were as follows: lecithin D, 2.91 per cent; lecithin C, 2.12 per cent; lecithin E, 1.45 per cent; and lecithin A, 1.43 per cent. The differences in the actions of lecithin solutions E and A were negligible. Lecithin J, prepared by simple alcoholic extraction of crude egg lecithin, showed slightly less activity than lecithin G, 3.36 per cent. The crude egg lecithin extract had a high phosphorus content, the major portion of which was undoubtedly present in substances other than lecithin. Therefore, the exact amount of lecithin could not be determined.

The results of these experiments, with the exception of lecithin J, suggest that the capacity of lecithin to improve the titration picture of oversensitive antigens is a function of concentration and apparently is independent of whether the lecithin is prepared from beef heart muscle or from soy bean.

5. *The sensitivity and specificity adjustments of oversensitive antigens corrected with lecithin.* The change in the titration picture as a result of the addition of lecithin, as listed in Tables 1, 2 and 4, served as the indicator of the exact amount of lecithin to be added to a given antigen for comparative sensitivity studies with standard Kahn antigen. Each antigen to which lecithin was added was tested with weakly positive syphilitic serums and the results compared with those obtained with standard Kahn antigen. On the basis of these tests with serum, final adjustments for sensitivity were made, when necessary, by the addition of cholesterolized alcohol or by small changes of the titers. An illustration of this procedure with antigen O₃ is given in Table 5.

Table 5A shows that a 2 per cent dilution of antigen O₃ with lecithin solution B was sufficient to give an acceptable titration picture with a titer of 1 + 1.3. According to Table 5B, however, the reactions with syphilitic serums were not quite as sensitive as those with standard Kahn antigen. When the tests were repeated employing a reduced titer of 1 + 1.25, the results were found to be closely comparable to those of standard antigen.

Similar adjustments were carried out on each of the other antigens and the final methods of correction are given in Table 6.

Antigen O₁ was corrected by 15 per cent dilution with lecithin solution F plus 10 per cent dilution with cholesterolized alcohol and used at a titer of 1 + 1.3. Antigen O₂ was corrected by 5 per cent dilution with lecithin solution C and used

TABLE 4
EFFECT OF VARIOUS CONCENTRATIONS OF LECITHIN ON THE TITRATION
OF AN OVERSENSITIVE ANTIGEN

LECITHIN SOLUTION		AMOUNTS IN ML. OF ANTIGEN AND SALT SOLUTION	TITRATION READINGS OF ANTIGEN O ₂ DILUTED WITH VARIOUS PERCENTAGES OF DIFFERENT LECITHIN SOLUTIONS		
Designation	Concentration, per cent		1 per cent	5 per cent	10 per cent
H	4.98	1 + 1.1	Tr. cloudy	Sl. too clear	Too clear
		1 + 1.3	Tr. cloudy	Too clear	Watery clear
		1 + 1.5	Tr. clear	Watery clear	Watery clear
		1 + 1.7	Sl. clear	Watery clear	Watery clear
G	3.36	1 + 1.1	Opalescent	Opalescent	Sl. too clear
		1 + 1.3	Cloudy, fuzzy	Too clear	Too clear
		1 + 1.5	Tr. cloudy	Watery clear	Watery clear
		1 + 1.7	Tr. clear	Watery clear	Watery clear
D	2.91	1 + 1.1	Sl. cloudy	Sl. cloudy	Tr. cloudy
		1 + 1.3	Tr. cloudy	Tr. cloudy	Watery clear
		1 + 1.5	Tr. cloudy	Opalescent	Watery clear
		1 + 1.7	Tr. cloudy	Sl. too clear	Watery clear
C	2.12	1 + 1.1	Tr. cloudy	Tr. cloudy	Tr. cloudy
		1 + 1.3	Sl. cloudy	Opalescent	Opalescent
		1 + 1.5	Sl. cloudy	Opalescent	Opalescent
		1 + 1.7	Sl. clear	Sl. clear	Sl. clear
E	1.45	1 + 1.1	Tr. cloudy	Sl. cloudy	Sl. cloudy
		1 + 1.3	Sl. cloudy	Sl. cloudy	Tr. cloudy
		1 + 1.5	Sl. cloudy	Sl. cloudy	Opalescent
		1 + 1.7	Sl. cloudy	Tr. cloudy	Sl. too clear
A	1.43	1 + 1.1	Sl. cloudy	Sl. cloudy	Tr. cloudy
		1 + 1.3	Sl. cloudy	Sl. cloudy	Tr. cloudy
		1 + 1.5	Sl. cloudy	Tr. cloudy	Tr. cloudy
		1 + 1.7	Tr. cloudy	Tr. cloudy	Tr. clear
J*		1 + 1.1	Sl. cloudy	Tr. cloudy	Sl. clear
		1 + 1.3	Sl. cloudy	Sl. clear	Too clear
		1 + 1.5	Sl. cloudy	Watery clear	Watery clear
		1 + 1.7	Tr. cloudy	Watery clear	Watery clear
0.6 per cent cholesterolized alcohol		1 + 1.1	Tr. cloudy	Tr. cloudy	Opalescent
		1 + 1.3	Sl. cloudy	Sl. cloudy	Tr. clear
		1 + 1.5	Cloudy	Sl. cloudy	Tr. cloudy
		1 + 1.7	Cloudy	Sl. cloudy	Tr. cloudy

* Lecithin J (8.48%P), a colored alcoholic extract of commercial egg-lecithin.

at a 1 + 1.25 titer. As given above, antigen O₂ was corrected by 2 per cent dilution with lecithin solution B and used at a titer of 1 + 1.25. In addition,

antigen O_3 was corrected by 3 per cent dilution with crude lecithin extract J plus 3 per cent dilution with cholesterolized alcohol and used at a titer of 1 + 1.3. This antigen will be referred to as O_{3J} .

TABLE 5

ILLUSTRATION OF THE SENSITIVITY ADJUSTMENT OF AN OVERSENSITIVE ANTIGEN CORRECTED WITH LECITHIN

A. Determination of titer according to the opalescence of antigen suspension

ANTI-GEN	AMOUNTS IN ML. OF ANTIGEN AND SALT SOLUTION	TITRATION READINGS OF ANTIGEN DILUTED WITH VARIOUS PERCENTAGES OF LECITHIN SOLUTION B			
		Undiluted	2 per cent	5 per cent	10 per cent
O_3	1 + 1.1	Tr. cloudy	Tr. cloudy	Sl. too clear	Too clear
	1 + 1.3	Sl. cloudy	Opalescent†	Watery clear	Watery clear
	1 + 1.5	Cloudy, fuzzy	Sl. clear	Watery clear	Watery clear
	1 + 1.7	Cloudy	Sl. too clear	Watery clear	Watery clear

† Titer.

B. Adjustment of titer according to antigen sensitivity with syphilitic sera

SERUM	ANTIGEN O_3 2 PER CENT DILUTION LECITHIN B TITER 1 + 1.3	STANDARD ANTIGEN (CONTROL) TITER 1 + 1.2	ANTIGEN O_3 2 PER CENT DILUTION LECITHIN B TITER 1 + 1.25	STANDARD ANTIGEN (CONTROL) TITER 1 + 1.2
1	— — —*	— — —*	— — —*	— — —*
2	— ± 4	— ± 4	— ± 4	— ± 4
3	— ± —	— — —	— ± 4	— ± 4
4	— ± 4	— ± 4	— 2 4	— 4 4
5	— — —	— ± 4	± 4 4	± 4 4

* The negative reactions appeared opalescent.

TABLE 6

METHODS OF FINAL CORRECTION FOR OVERSENSITIVE ANTIGENS

ANTIGEN	ADJUSTMENT WITH LECITHIN AND CHOLESTEROLIZED ALCOHOL	TITER (ML. OF ANTIGEN AND SALT SOLUTION)
O_1	15 per cent dilution lecithin solution F 10 per cent dilution cholesterolized alcohol	1 + 1.3
O_2	5 per cent dilution lecithin solution C	1 + 1.25
O_3	2 per cent dilution lecithin solution B	1 + 1.25
O_{3J}	3 per cent dilution lecithin solution J 3 per cent dilution cholesterolized alcohol	1 + 1.3

6. *The use of antigens corrected with lecithin in the Kahn differential temperature and triple quantitative technics.* In the Kahn verification test,¹ the technics for the differentiation between syphilitic and nonsyphilitic reactions include the differential temperature and triple quantitative technics. In the differential

temperature technic, three Kahn tests are performed on each serum, one at 1 C., another at room temperature and the other at 37 C. In the triple quantitative technic, three quantitative Kahn tests are performed on each serum using a serial

TABLE 7

OVERSENSITIVE ANTIGENS CORRECTED TO STANDARD SENSITIVITY BY THE ADDITION OF LECITHIN IN THE KAHN DIFFERENTIAL TEMPERATURE TECHNIC

SERUM	ANTIGEN O ₁ 1 + 1.3	ANTIGEN O ₂ 1 + 1.25	ANTIGEN O ₂ 1 + 1.25	ANTIGEN O ₃ 1 + 1.3	STANDARD ANTIGEN (CONTROL) 1 + 1.2
Results at 1 C.					
1	— — —	— — —	— — —	— — —	— — —
2	— 4 4	— 1 4	± 4 4	— 3 4	— 1 4
3	— 2 4	— 1 4	± 4 4	— 2 4	— 1 4
4	± 4 4	— 3 4	± 4 4	± 4 4	— 3 4
5	— 4 4	— ± 4	2 4 4	— 1 4	— ± 3
6	— 4 4	± 4 4	1 4 4	1 4 4	± 2 4
7	— 4 4	± 4 4	2 4 4	— 4 4	± 2 4
8	— ± 4	— 2 4	± 4 4	± 4 4	— ± 1
9	— ± 4	— 1 2	1 4 4	— ± 4	— ± 1
10	— — —	— — —	— — —	— — —	— — —
Results at room temperature					
1	— — —	— — —	— — —	— — —	— — —
2	— 4 4	— 4 4	— 4 4	— 4 4	— 4 4
3	1 4 4	± 4 4	1 4 4	1 4 4	1 4 4
4	2 4 4	± 4 4	2 4 4	2 4 4	2 4 4
5	± 4 4	± 4 4	— 4 4	— 4 4	— 4 4
6	± 4 4	± 4 4	— 4 4	— 4 4	— 4 4
7	— 4 4	± 4 4	1 4 4	— 4 4	— 4 4
8	1 4 4	— 4 4	— 4 4	— 4 4	— 4 4
9	— 4 4	— 3 4	— 3 4	— 4 4	— 4 4
10	— — —	— — —	— — —	— — —	— — —
Results at 37 C.					
1	— — —	— — —	— — —	— — —	— — —
2	4 4 4	4 4 4	3 4 4	3 4 4	4 4 4
3	4 4 4	4 4 4	4 4 4	4 4 4	4 4 4
4	4 4 4	4 4 4	4 4 4	4 4 4	4 4 4
5	4 4 4	4 4 4	4 4 4	4 4 4	4 4 4
6	4 4 4	4 4 4	4 4 4	4 4 4	4 4 4
7	4 4 4	4 4 4	4 4 4	4 4 4	4 4 4
8	4 4 4	4 4 4	4 4 4	4 4 4	4 4 4
9	4 4 4	4 4 4	3 4 4	4 4 4	4 4 4
10	— — —	— — —	— — —	— — —	— — —

dilution of serum made, respectively, with distilled water, 0.9 per cent sodium chloride solution and 2.5 per cent sodium chloride solution. Antigens O₁, O₂, O₃ and O₃J were each employed in these two technics in comparison with Kahn standard antigen.

The results obtained with ten pooled syphilitic serums in the differential temperature technic are given in Table 7. An examination of this table shows that the results with antigens O₁, O₂, O₃ and O₃J are broadly similar to one another and to the control. In the tests performed at room temperature and 37 C., the reactions obtained with the corrected antigens are practically identical with those

TABLE 8
OVERSENSITIVE ANTIGENS CORRECTED TO STANDARD SENSITIVITY BY THE ADDITION OF LECITHIN IN THE KAHN TRIPLE QUANTITATIVE TECHNIC

ANTIGEN*	SERUM DILUTION	SERIAL DILUTIONS OF SERUM PREPARED WITH VARIOUS PERCENTAGES OF SODIUM CHLORIDE SOLUTION					
		Serum 1			Serum 2		
		0 per cent	0.9 per cent	2.5 per cent	0 per cent	0.9 per cent	2.5 per cent
O ₁	1:2.5	4	4	4	4	4	4
	1:5	3	4	4	±	—	4
	1:10	1	±	4	—	—	4
	1:20	±	—	4	—	—	4
	1:40	—	—	4	—	—	1
O ₂	1:2.5	4	4	4	3	4	4
	1:5	4	4	4	±	±	4
	1:10	±	±	4	—	—	3
	1:20	—	—	4	—	—	1
	1:40	—	—	3	—	—	±
O ₃	1:2.5	4	4	4	4	4	4
	1:5	4	4	4	±	1	4
	1:10	4	4	4	1	—	4
	1:20	2	—	4	1	—	2
	1:40	4	—	4	3	—	—
O ₃ J	1:2.5	4	4	4	4	4	4
	1:5	4	4	4	±	±	4
	1:10	2	4	4	—	—	4
	1:20	±	—	4	—	—	4
	1:40	±	—	4	—	—	1
Standard antigen (control)	1:2.5	4	4	4	4	4	4
	1:5	4	4	4	±	±	4
	1:10	2	4	4	—	—	4
	1:20	1	—	4	—	—	2
	1:40	1	—	4	—	—	1

* Titers same as in Table 7.

of the control antigen, while at 1 C. the reactions are slightly stronger than the control. Antigens O₁, O₂ and O₃J gave progressively stronger reactions with increased temperatures which is the typical reaction with syphilitic serums. Antigen O₃ gave stronger reactions at 1 C. than at room temperature which may occasionally occur without seriously affecting the interpretation of the test, pro-

vided the reactions at 37 C. are stronger than those given at either 1 C. or room temperature.

The results of the triple quantitative procedures, employing the four corrected antigens and a control standard antigen with two syphilitic serums, are presented in Table 8. It is evident that the quantitative reactions given by the corrected antigens are similar to those given by the control antigen. A typical syphilitic type of reaction is observed with each antigen, showing weakest precipitation in the serum dilutions with water, stronger in the dilutions with 0.9 per cent sodium chloride and strongest in the dilutions with 2.5 per cent sodium chloride. An additional verification technic, the salt dispersibility of the precipitates, was applied to the differential temperature and triple quantitative technics. It was found that the precipitates, both of the corrected antigen tests and control tests, were nondispersible, a characteristic of precipitates with syphilitic serums.

SUMMARY

Studies on the effect of lecithin on Kahn antigen led to several observations. Lecithin has a marked effect on both the titer and sensitivity of the antigen. It reduces the amount of salt solution necessary in the titer and it reduces the sensitivity of the antigen with syphilitic serums. An antigen showing no titration end point (because of nondispersible aggregates throughout the titration range) and outside the range of correction by the usual methods, may be so modified by lecithin as to give a desirable titer and correct sensitivity. The degree of reduction in sensitivity of an antigen is directly related to the concentration of lecithin added. The origin of lecithin, as to whether it is prepared from beef heart, soy bean or egg yolk, is apparently of no significance insofar as its effect on the antigen is concerned. Oversensitive antigens corrected with lecithin were found to behave like standard antigen, not only in the standard Kahn test, but also in the differential temperature and triple quantitative technics of the verification test. Lecithin added to undersensitive antigens did not markedly alter the titer nor the sensitivity. Choline hydrochloride added to either oversensitive or undersensitive antigen behaved somewhat similarly but less effectively than lecithin.

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THE EFFECT OF LIPIDS ON KAHN ANTIGEN

II. INCREASE OF SENSITIVITY BY THE ADDITION OF CEPHALIN*

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In the preceding article,⁵ it was shown that lecithin added to Kahn antigen reduces its sensitivity with syphilitic serums. This observation was found to have practical application in correcting oversensitive antigens to the sensitivity of standard Kahn antigen by the addition of appropriate amounts of lecithin. The question arose as to whether it would be possible to correct undersensitive antigens to standard requirements by adding some lipid which possessed the property of increasing antigen sensitivity. The use of cephalin suggested itself for trial. Accordingly, an attempt was made to extract cephalin from commercial soy bean lecithin, beef heart muscle and from crude sheep brain cephalin. The resulting phospholipid solutions were added in various amounts to antigens of different sensitivities and the effects studied. Data will be herewith presented indicating that cephalin increases the sensitivity of undersensitive antigens and that this lipid may be employed in correcting such antigens to the requirements of standard Kahn antigen.

MATERIAL AND METHODS

Antigens. Three undersensitive antigens, U_1 , U_2 and U_3 , and three oversensitive antigens, O_1 , O_2 and O_3 , were employed in this study.

Cephalin prepared from "Centrol Soya Lecithin." The following method was employed: Fifty gm. of the crude lecithin were extracted with 1 liter of hot absolute methyl alcohol and filtered. The filtrate was kept overnight in the ice box and the crystalline precipitate was filtered off. A 50 per cent aqueous solution of cadmium chloride was added to the filtrate until no more precipitation occurred. This material was stored overnight in the cold; it was then centrifuged and the precipitate washed several times with absolute methyl alcohol until the supernatant was almost colorless. The precipitate was dissolved in 200 ml. of petroleum ether and extracted thirty times with 25 to 50 ml. volumes of 80 per cent ethyl alcohol. The petroleum ether extract was distilled to dryness and the residue dissolved in 100 ml. of chloroform. Methyl alcohol saturated with ammonia was added to the chloroform solution until precipitation was complete. The solution was then shaken with 50 ml. of saturated sodium chloride solution. Normal hydrochloric acid was added until the aqueous phase was slightly acid. The chloroform was drawn off and shaken with saturated sodium chloride until the aqueous phase was neutral. Where necessary, 95 per cent ethyl alcohol was added to break the emulsion. The chloroform solution was concentrated

* Aided by a grant from the Difco Laboratories, Inc., Detroit. Received for publication, September 28, 1946.

almost to dryness by vacuum distillation, using a wood splint to prevent bumping. Fifty ml. of absolute ethyl alcohol were added to the residue. This solution was allowed to stand overnight at 21 C. It was then filtered and 0.6 per cent cholesterol was added. Three solutions of this cephalin, A, B and C, were available for study.

Cephalin prepared from beef heart muscle. The preliminary steps consisted in applying the technic for the isolation of lecithin described by Pangborn.⁴ An alcoholic extract of beef heart previously dried by acetone was precipitated with cadmium chloride and the precipitate extracted thirty times with a petroleum ether—80 per cent alcohol mixture (3:1). This extract was distilled to dryness and the residue dissolved in 100 ml. chloroform. The remainder of the purification process was the same as described above for the preparation of soy bean cephalin. Two solutions of this cephalin, D and E, were available for study.

Cephalin prepared from commercial sheep brain cephalin (Difco). A solution of cephalin was prepared by dissolving 10 gm. of the reddish brown powder in 40 ml. of anesthesia ether. Then 100 ml. of hot 0.6 per cent cholesterolized absolute alcohol was added, the mixture was thoroughly shaken and allowed to cool. It was filtered and the precipitate discarded. This solution was heated in an open flask until no ether odor was detectable. It was refiltered and made up to a volume of 100 ml. with noncholesterolized absolute alcohol. This solution was designated as F. The concentrations of the cephalin solutions were as follows:

DESIGNATION	CEPHALIN CONCENTRATION* IN PER CENT	SOURCE
A	2.7	Soy bean
B	0.6	1 part of A plus 4 parts cholesterolized alcohol
C	0.9	Soy bean
D	2.8	Beef heart (50 per cent concentrate of E)
E	1.4	Beef heart
F	2.1	Sheep brain

* Calculated for P = 3.8 per cent in cephalin.

It should be added that we do not consider the above cephalin preparations to be of high purity. Indeed, we consider them to be cephalin-like compounds only. Preliminary experiments indicated that these preparations tended to increase the sensitivity of undersensitive Kahn antigen and hence, were utilized in this study.

Method of antigen titration. This technic has been described in the preceding article.⁵

Method of antigen correction. The technical procedure for the correction of antigens to standard sensitivity is essentially the same as presented in the preceding article. Ten ml. samples of antigens were diluted with 1 per cent, 5 per cent and 10 per cent, respectively, of a given cephalin solution and titrated with 0.9 per cent sodium chloride solution. After an acceptable titer was obtained, the sensitivity of the antigen was tested with syphilitic and nonsyphilitic serums, employing Kahn standard antigen as a control.

Further adjustments were made when necessary, either by increasing or de-

creasing the amount of cephalin added to the antigen, or by the use of established methods in antigen standardization, considered in the preceding article. Antigens corrected to the sensitivity of standard Kahn antigen were then applied to the supplementary Kahn procedures.

EXPERIMENTAL

1. *Effect of cephalin on the titration picture of undersensitive antigens.* Three undersensitive antigens, U_1 , U_2 and U_3 were diluted with 1 per cent, 5 per cent and 10 per cent of cephalin solution B. Titrations were performed on the diluted antigens employing 0.9 per cent sodium chloride solution. Control titrations were made on these antigens with corresponding dilutions with cholesterolized alcohol. The results of this experiment are given in Table 1.

An examination of Table 1 shows that an addition of as little as 1 per cent of cephalin solution B to antigen U_1 is sufficient to change its titration picture. A cloudy zone is apparent with the increase in salt solution upon the addition of 1 per cent and 5 per cent of the cephalin solution. With the 10 per cent addition, cloudiness persists throughout the titration, indicative of marked oversensitivity. The dilutions with cholesterolized alcohol improved the titration picture of antigen U_1 but were not sufficient to give a workable titer. Antigen U_2 behaved somewhat similarly to U_1 and the 10 per cent dilution with cephalin gave a titration picture suggesting marked oversensitivity. Antigen U_3 , which gave a workable titer in its original state, although not the desired titration picture, was markedly affected by the addition of cephalin. As little as 1 per cent dilution was sufficient to give heavy aggregates in all tubes, indicating high oversensitivity. This antigen could be corrected to standard sensitivity by the addition of cholesterolized alcohol without, however, improving the titration picture.

2. *Effect of cephalin on the titration picture of oversensitive antigens.* Portions of oversensitive antigens O_1 , O_2 and O_3 were diluted with 1 per cent, 5 per cent and 10 per cent of cephalin solution B and with cholesterolized alcohol as controls. Titrations were carried out on these samples in the usual manner. The results are summarized in Table 2.

It is apparent from Table 2 that the titration pictures of oversensitive antigens diluted with cephalin solution A change in the direction of even greater oversensitivity and show heavy aggregates throughout the titration zone. Dilution of the antigens with cholesterolized alcohol did not alter the titration picture significantly.

3. *Effect of various cephalin preparations on the titration picture of undersensitive antigen.* Previous experiments showed⁵ that the ability of lecithin solutions to reduce the turbidity in titrations of oversensitive antigen increased with increasing concentrations of lecithin; also that the source of lecithin was apparently unimportant. Corresponding experiments were carried out with cephalin solutions and undersensitive antigens.

Five cephalin solutions were employed: solution A (2.7 per cent) and solution C (0.9 per cent) from soy bean, solution D (2.8 per cent) and solution E (1.4 per cent) from beef heart, and cephalin solution F (2.1 per cent) from sheep brain.

TABLE I
ILLUSTRATION OF THE EFFECT OF CEPHALIN ON THE TITRATION PICTURE OF UNDERSENSITIVE ANTIGENS

AMOUNTS IN ML. OF ANTIGEN AND SALT SOLUTION	TITRATION READINGS OF UNCORRECTED ANTIGENS	TITRATION READINGS OF ANTIGENS DILUTED WITH VARIOUS PERCENTAGES OF CEPHALIN SOLUTION II				TITRATION READINGS OF ANTIGENS DILUTED WITH VARIOUS PERCENTAGES OF CHOLESTEROLIZED ALCOHOL (CONTROL)			
		1 per cent	5 per cent	10 per cent		1 per cent	5 per cent	10 per cent	
Antigen U ₁									
1 + 1.1	Sl. too clear	Tr. cloudy	Cloudy	Cloudy	Sl. too clear	Sl. too clear	Sl. clear		
1 + 1.3	Watery clear	Tr. clear	Cloudy	Cloudy	Watery clear	Too clear	Sl. too clear		
1 + 1.5	Watery clear	Opalescent	Sl. cloudy	Cloudy	Watery clear	Watery clear	Too clear		
1 + 1.7	Watery clear	Tr. cloudy	Sl. cloudy	Cloudy	Watery clear	Watery clear	Watery clear		
Antigen U ₂									
1 + 1.1	Tr. clear	Tr. clear	Sl. clear	Cloudy	Tr. clear	Opalescent	Opalescent		
1 + 1.3	Sl. too clear	Sl. too clear	Sl. clear	Cloudy	Sl. too clear	Too clear	Sl. too clear		
1 + 1.5	Watery clear	Too clear	Tr. cloudy	Cloudy	Watery clear	Too clear	Too clear		
1 + 1.7	Watery clear	Watery clear	Tr. cloudy	Sl. cloudy	Watery clear	Watery clear	Watery clear		
Antigen U ₃									
1 + 1.1	Tr. cloudy	Heavy agg.	Heavy agg.	Heavy agg.	Tr. cloudy	Sl. cloudy	Sl. cloudy		
1 + 1.3	Opalescent	Heavy agg.	Heavy agg.	Heavy agg.	Opalescent	Opalescent	Opalescent		
1 + 1.5	Opalescent	Heavy agg.	Heavy agg.	Heavy agg.	Opalescent	Opalescent	Opalescent		
1 + 1.7	Sl. clear	Heavy agg.	Heavy agg.	Heavy agg.	Sl. clear	Sl. clear	Opalescent		

TABLE 2
ILLUSTRATION OF THE EFFECT OF CEPHALIN ON THE TITRATION PICTURE OF OVERSENSITIVE ANTIGENS

AMOUNTS IN ML. OF ANTIGEN AND SALT SOLUTION	TITRATION READINGS OF UNCORRECTED ANTIGENS	TITRATION READINGS OF ANTIGENS DILUTED WITH VARIOUS PERCENTAGES OF CEPHALIN SOLUTION B				TITRATION READINGS OF ANTIGENS DILUTED WITH VARIOUS PERCENT- AGES OF CHOLESTEROLIZED ALCOHOL (CONTROL)			
		1 per cent				1 per cent			
		5 per cent				5 per cent			
Antigen O ₁									
1 + 1.1	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.
1 + 1.3	Cloudy	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.	Cloudy	Cloudy, agg.	Cloudy, agg.	Cloudy, agg.
1 + 1.5	Sl. cloudy	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.	Sl. cloudy	Cloudy	Cloudy	Cloudy
1 + 1.7	Sl. clear	Cloudy, agg.	Heavy agg.	Heavy agg.	Heavy agg.	Sl. clear	Tr. cloudy	Tr. cloudy	Tr. cloudy
Antigen O ₂									
1 + 1.1	Cloudy, agg.	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.	Cloudy, agg.	Sl. cloudy	Tr. cloudy	Tr. cloudy
1 + 1.3	Cloudy, agg.	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.	Cloudy, agg.	Cloudy, agg.	Heavy agg.	Heavy agg.
1 + 1.5	Opalescent	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.	Opalescent	Opalescent	Sl. cloudy	Sl. cloudy
1 + 1.7	Too clear	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.	Too clear	Too clear	Sl. clear	Sl. clear
Antigen O ₃									
1 + 1.1	Tr. cloudy	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.	Tr. cloudy	Tr. cloudy	Opalescent	Opalescent
1 + 1.3	Sl. cloudy	Heavy agg.	Heavy agg.	Heavy agg.	Heavy agg.	Sl. cloudy	Tr. cloudy	Tr. clear	Tr. clear
1 + 1.5	Cloudy, fuzzy	Cloudy, agg.	Heavy agg.	Heavy agg.	Heavy agg.	Cloudy, fuzzy	Sl. cloudy	Tr. cloudy	Tr. cloudy
1 + 1.7	Cloudy	Cloudy, agg.	Heavy agg.	Heavy agg.	Heavy agg.	Cloudy	Sl. cloudy	Tr. cloudy	Tr. cloudy

Antigen U₁ was diluted with 1 per cent, 5 per cent and 10 per cent of each of these solutions. Controls with 0.6 per cent cholesterolized alcohol were also employed. Titration results are shown in Table 3.

Although the number of cephalin solutions is limited, it appears from the data in Table 3 that two factors, source and phosphorus concentration, play a part in

TABLE 3

EFFECT OF VARIOUS CONCENTRATIONS OF SOY BEAN AND BEEF HEART CEPHALIN ON THE TITRATION PICTURE OF AN UNDERSENSITIVE ANTIGEN

CEPHALIN SOLUTION			AMOUNTS IN ML. OF ANTIGEN AND SALT SOLUTION	TITRATION READINGS OF ANTIGEN U ₁ DILUTED WITH VARIOUS PERCENTAGES OF DIFFERENT CEPHALIN SOLUTIONS		
Designation	Source	Concentration, per cent		1 per cent	5 per cent	10 per cent
A	Soybean	2.7	1 ÷ 1.1	Tr. cloudy	Cloudy, fuzzy	Cloudy, agg.
			1 ÷ 1.3	Tr. clear	Cloudy, fuzzy	Cloudy, agg.
			1 ÷ 1.5	Opalescent	Cloudy, fuzzy	Cloudy, agg.
			1 ÷ 1.7	Tr. cloudy	Cloudy, fuzzy	Cloudy, agg.
C	Soybean	0.9	1 ÷ 1.1	Sl. clear	Tr. cloudy	Cloudy, agg.
			1 ÷ 1.3	Sl. too clear	Tr. clear	Cloudy, agg.
			1 ÷ 1.5	Too clear	Tr. cloudy	Cloudy, agg.
			1 ÷ 1.7	Watery clear	Tr. cloudy	Sl. cloudy
D	Beef heart	2.8	1 ÷ 1.1	Sl. too clear	Tr. cloudy	Heavy agg.
			1 ÷ 1.3	Too clear	Tr. cloudy	Heavy agg.
			1 ÷ 1.5	Watery clear	Sl. cloudy	Heavy agg.
			1 ÷ 1.7	Watery clear	Cloudy, agg.	Heavy, agg.
E	Beef heart	1.4	1 ÷ 1.1	Tr. clear	Opalescent	Opalescent
			1 ÷ 1.3	Too clear	Sl. clear	Tr. clear
			1 ÷ 1.5	Watery clear	Sl. too clear	Tr. cloudy
			1 ÷ 1.7	Watery clear	Too clear	Tr. cloudy
F	Sheep brain	2.1	1 ÷ 1.1	Tr. cloudy	Sl. cloudy	Cloudy
			1 ÷ 1.3	Too clear	Tr. cloudy	Cloudy
			1 ÷ 1.5	Watery clear	Too clear	Tr. cloudy
			1 ÷ 1.7	Watery clear	Too clear	Sl. cloudy
0.6 per cent cholesterol- ized alcohol			1 ÷ 1.1	Sl. too clear	Sl. too clear	Sl. clear
			1 ÷ 1.3	Watery clear	Too clear	Sl. too clear
			1 ÷ 1.5	Watery clear	Watery clear	Too clear
			1 ÷ 1.7	Watery clear	Watery clear	Watery clear

determining the activity of cephalin, the latter being taken as a measure of the cephalin concentration. If the activities of the two soy bean extracts or the two beef heart extracts are compared to each other, then in either case the solution containing the higher concentration of cephalin, solution A or D, causes heavier aggregation in the titration picture than does the preparation containing the lower cephalin concentration, solution C or E.

In a further comparison made between soy bean cephalin A (2.7 per cent) and beef heart cephalin D (2.8 per cent) it appears, especially in the 1 per cent dilutions, that the former is somewhat more active than the latter. This greater activity of the soy bean product over the beef heart product becomes more apparent when the titration pictures with soy bean cephalin C (0.9 per cent) and beef heart cephalin E (1.4 per cent) are compared. The soy bean extract is considerably more active although its cephalin content is only about 70 per cent of that of the beef heart solution.

On the basis of the cephalin concentrations and the titration pictures obtained with the sheep brain cephalin solutions, it is difficult to determine whether its effect on undersensitive antigen is more closely comparable to that of the beef heart cephalin or to the soy bean product. It is apparent, however, that the sheep brain cephalin solution increases the aggregation in the titration, which is indicative of increased sensitivity.

4. *The sensitivity and specificity adjustments of undersensitive antigens corrected with cephalin.* In the preliminary steps for the correction of undersensitive antigens, titrations were carried out on samples which had been diluted with 1 per cent, 5 per cent and 10 per cent of various cephalin solutions. The results of these trials indicated the more exact dilutions necessary for an acceptable titer and titration picture. When these were arrived at, the antigens were tested against weakly positive syphilitic serums. On the basis of these sensitivity tests, final adjustments were made by dilutions with cholesterolized alcohol or by small changes in the titer. An illustration of these steps in antigen correction is presented in Table 4 with antigen U_2 .

Table 4A shows that dilution of antigen U_2 with 2 per cent of cephalin solution B gives an acceptable titration picture and that dilution with 3 per cent gives a workable titer but a rather flat titration picture. In either case, the titer would be approximately $1 + 1.1$. It is seen in part B of this table that serum tests with the antigen diluted with 2 per cent of the cephalin solution at the titer $1 + 1.15$ and with the antigen diluted with 3 per cent of the cephalin at $1 + 1.2$ were undersensitive, and the negatives appeared too clear. The antigen diluted with 3 per cent of the cephalin solution was retested with serums employing titers of $1 + 1.15$ and $1 + 1.1$. The results were still undersensitive and the negatives slightly too clear. A second check was made on this antigen at a titer of $1 + 1.1$ after it had been diluted with 10 per cent of cholesterolized alcohol. In this case (serums 11 to 15 in the table), the opalescence of the negatives was satisfactory and the sensitivity results were similar to those obtained with standard Kahn antigen.

Each antigen was thus adjusted to standard sensitivity and the final methods of corrections are given in Table 5.

In correcting antigen U_1 , both cephalin and lecithin were employed. Since one lipid increases and the other decreases sensitivity, it was found possible to offset some excess of one lipid by the addition of the other. This antigen was corrected by dilution with 15 per cent of cephalin B plus 4 per cent of lecithin B plus 10 per cent of cholesterolized alcohol; the titer was $1 + 1.3$. Antigen U_2

was corrected, as indicated above, by dilution with 3 per cent of cephalin B and 10 per cent of cholesterolized alcohol; the titer was $1 + 1.1$. Antigen U_3 was borderline in sensitivity and cephalin was not found helpful. It was corrected, by 5 per cent cholesterolized alcohol and 1 per cent lecithin solution C; the titer was $1 + 1.3$.

TABLE 4

SENSITIVITY ADJUSTMENT OF AN UNDERSENSITIVE ANTIGEN CORRECTED WITH CEPHALIN
A. Determination of titer according to the opalescence of the antigen suspension

AMOUNTS IN ML. OF ANTI- GEN AND SALT SOLUTION	TITRATION READINGS OF UN- CORRECTED ANTIGEN	TITRATION READINGS OF ANTIGEN U_2 DILUTED WITH VARIOUS PERCENTAGES OF CEPHALIN SOLUTION B		
		1 per cent	2 per cent	3 per cent
$1 + 1.1$	Tr. clear	Tr. clear	Opalescent	Opalescent
$1 + 1.3$	Sl. too clear	Sl. too clear	Sl. too clear	Tr. clear
$1 + 1.5$	Watery clear	Too clear	Too clear	Tr. clear
$1 + 1.7$	Watery clear	Watery clear	Watery clear	Sl. clear

B. Adjustment of titer according to antigen sensitivity with syphilitic serums

SERUM NUMBER	2 PER CENT CEPHALIN B	3 PER CENT CEPHALIN B	STANDARD ANTIGEN (CONTROL)
	Titer $1 + 1.15$	Titer $1 + 1.2$	Titer $1 + 1.2$
1	— — 1	— 1 3	— 4 4
2	— 4 4	— 3 4	3 4 4
3	2 4 4	3 4 4	4 4 4
4	— \pm 4	— \pm 4	— 4 4
5	— — —	— — —	— — —
		Titer $1 + 1.15$	Titer $1 + 1.2$
6		— — 4	— — 4
7		— 1 4	— 4 4
8		— 2 4	\pm 4 4
9		— 2 4	— 4 4
10		— — —	— — —
		Titer $1 + 1.1$	Titer $1 + 1.2$
11		\pm 4 4	\pm 4 4
12		\pm 4 4	\pm 4 4
13		— 4 4	— 4 4
14		— 2 4	— 4 4
15		— — —*	— — —

* Negative reactions opalescent.

Another sample of antigen U_1 was corrected by adding 3 per cent of commercial cephalin solution F (instead of purified cephalin) and 25 per cent of cholesterolized alcohol. The titer of this antigen was $1 + 1.3$, and it shall be referred to hereafter as antigen U_1F .

5. *The use of antigens corrected with cephalin in the Kahn differential temperature and triple quantitative technics of the verification test.* The technics for carrying

out these tests were exactly the same as those described in the preceding paper⁵ in respect to oversensitive antigens.

Ten pooled syphilitic serums were tested in the differential temperature technic with corrected antigens, U_1 , U_1F , U_2 and U_3 , alongside a control with standard Kahn antigen. The results of these tests are summarized in Table 6.

If the reactions of the ten serums with antigens U_1 , U_1F , U_2 , U_3 and standard antigen control are compared, it will be observed that at each temperature the results are almost identical, indicating that the sensitivity of the corrected antigens is the same as that of an approved lot of Kahn antigen. No significant differences occurred in the reactions with antigen U_1 , corrected with cephalin solution B, and with antigen U_1F , corrected with commercial cephalin solution F.

If the comparisons are made of the reactions with each antigen at 1 C., room temperature and 37 C., it will be noted that the reactions at the first two temperatures are about the same and some reactions at 37 C. are somewhat stronger

TABLE 5
METHODS OF FINAL CORRECTION OF UNDERSENSITIVE ANTIGENS

ANTIGEN	ADJUSTMENT BY DILUTION WITH:	TITER
U_1	15 per cent cephalin solution B 4 per cent lecithin solution B 10 per cent cholesterolized alcohol	1 + 1.3
U_2	3 per cent cephalin solution B 10 per cent cholesterolized alcohol	1 + 1.1
U_3^*	1 per cent lecithin solution C 5 per cent cholesterolized alcohol	1 + 1.3
U_1F	3 per cent cephalin solution F 25 per cent cholesterolized alcohol	1 + 1.3

* Borderline in sensitivity.

It is not uncommon to find that pooled syphilitic serums show similar reactivity at the three temperatures, a situation in which the salt dispersibility technic aids in the differentiation between syphilitic and nonsyphilitic reactions.

As a further check on the sensitivity of the corrected antigens, they were employed in the triple quantitative procedure with two syphilitic serums. The same serums were used with control antigen, Lot 85. The results of these tests are presented in Table 7.

It is seen from the table that the reactions of both serums with antigens U_1 , U_1F , U_2 and U_3 are similar to those of the control tests. The variations are insignificant.

6. *The antagonistic effect of lecithin and cephalin on antigens.* In Table 5 it was shown that the correction of antigen U_1 was brought about by the employment of lecithin in addition to cephalin and cholesterolized alcohol. It seemed worthwhile to perform an experiment showing more clearly the antagonistic

effect of cephalin and lecithin on an oversensitive and on an undersensitive antigen. A sample of each was first diluted with 10 per cent of cephalin solution

TABLE 6

UNDERSENSITIVE ANTIGENS CORRECTED TO STANDARD SENSITIVITY BY THE ADDITION OF CEPHALIN AND LECITHIN IN THE KAHN DIFFERENTIAL TEMPERATURE TECHNIC

SERUM	ANTIGEN U ₁ 1 + 1.3	ANTIGEN U ₁ F* 1 + 1.3	ANTIGEN U ₂ 1 + 1.1	ANTIGEN U ₂ 1 + 1.3	STANDARD ANTI- GEN (CONTROL) 1 + 1.2
Results at 1 C.					
1	- - -	- - -	- - -	- - -	- - -
2	± 1 4	± 4 4	± 4 4	- ± 4	± 1 4
3	± 3 4	1 4 4	± 4 4	± 3 4	± 4 4
4	- ± 4	- 2 4	- ± 4	- ± 1	- ± 4
5	- 3 4	1 4 4	± 4 4	- 3 4	- 4 4
6	± 4 4	± 4 4	± 4 4	- - 1	± 4 4
7	± 4 4	± 4 4	± 4 4	- ± 4	± 4 4
8	4 4 4	4 4 4	3 4 4	4 4 4	4 4 4
9	- 1 4	- 2 4	- 1 4	- 1 4 ⁿ	- 1 4
10	- - -	- - -	- - -	- - -	- - -
Results at room temperature					
1	- - -	- - -	- - -	- - -	- - -
2	± 4 4	± 4 4	± 4 4	± 4 4	± 4 4
3	± 4 4	± 4 4	± 4 4	± 4 4	± 4 4
4	- ± 3	- ± 4	- ± 3	- ± 3	- ± 4
5	- 4 4	- 4 4	- 4 4	- 4 4	- 4 4
6	± 4 4	± 4 4	± 4 4	± 4 4	± 4 4
7	- 3 4	- 3 4	- 2 4	- 3 4	- 4 4
8	4 4 4	4 4 4	4 4 4	4 4 4	4 4 4
9	- 1 3	- 1 4	- 4 4	- ± 4	- 1 4
10	- - -	- - -	- - -	- - -	- - -
Results at 37 C.					
1	- - -	- - -	- - -	- - -	- - -
2	1 4 4	1 4 4	2 4 4	± 4 4	1 4 4
3	2 4 4	2 4 4	3 4 4	2 4 4	2 4 4
4	± 4 4	± 4 4	± 4 4	± 4 4	± 4 4
5	1 4 4	± 4 4	1 4 4	± 4 4	± 4 4
6	2 4 4	1 4 4	1 4 4	1 4 4	1 4 4
7	2 4 4	± 4 4	± 4 4	± 4 4	± 4 4
8	4 4 4	4 4 4	4 4 4	4 4 4	4 4 4
9	- 4 4	- 4 4	- ± 3	- 4 4	- 4 4
10	- - -	- - -	- - -	- - -	- - -

* Corrected with commercial cephalin F.

B and titrated in the usual manner. Then, this sample (antigen plus cephalin) was further diluted with 10 per cent of lecithin solution A, and retitrated. The results of these experiments are shown in Table 8.

It can be seen that with both the oversensitive and undersensitive antigens the aggregates in the titration were made heavier by the addition of cephalin, indicating increased sensitivity, and that these aggregates were markedly reduced by the addition of lecithin, indicating decreased sensitivity.

TABLE 7

UNDERSENSITIVE ANTIGENS CORRECTED TO STANDARD SENSITIVITY BY THE ADDITION OF CEPHALIN AND LECITHIN IN THE KAHN TRIPLE QUANTITATIVE TECHNIC

ANTIGEN	SERUM DILUTIONS	SERIAL DILUTIONS OF SERUM PREPARED WITH VARIOUS PERCENTAGES OF SODIUM CHLORIDE SOLUTION					
		Serum 1			Serum 2		
		0 per cent	0.9 per cent	2.5 per cent	0 per cent	0.9 per cent	2.5 per cent
U ₁	1:2.5	4	4	4	4	4	4
	1:5	4	4	4	—	—	4
	1:10	3	4	4	—	—	4
	1:20	—	±	4	—	—	3
	1:40	—	—	4	2	—	2
U ₁ F†	1:2.5	4	4	4	4	4	4
	1:5	4	4	4	1	1	4
	1:10	2	4	4	—	—	4
	1:20	±	—	4	—	—	4
	1:40	—	—	4	—	—	2
U ₂	1:2.5	4	4	4	4	4	4
	1:5	2	4	4	±	±	4
	1:10	1	4	4	—	—	4
	1:20	—	—	4	—	—	2
	1:40	—	—	4	±	—	1
U ₃	1:2.5	4	4	4	4	4	4
	1:5	4	4	4	1	±	4
	1:10	1	4	4	±	—	4
	1:20	2	±	4	1	—	3
	1:40						
Standard antigen (control)	1:2.5	4	4	4	4	4	4
	1:5	4	4	4	±	±	4
	1:10	2	4	4	—	—	4
	1:20	1	—	4	—	—	2
	1:40	1	—	4	—	—	1

* Titers same as in Table 6.

† Corrected with commercial cephalin F.

The antagonistic effect of cephalin and lecithin is of interest and should prove of practical value in antigen standardization. Recent studies with cardiolipin antigen^{1, 2, 3} indicate that the various lipids in an antigen must be present in definite proportions. The two highly purified phospholipids, cardiolipin and

lecithin, with cholesterol in the right proportions, give good sensitivity results with syphilitic serums, while in improper proportions, antagonistic effects may lead to negative results with the same serums. It thus seems reasonable to assume that the proportions of various phospholipids present in routinely prepared Kahn antigen would affect its titer and sensitivity. In practice, the antagonism of lecithin and cephalin may be utilized in the standardization of antigens. For example, a freshly prepared antigen extract is found to be oversensitive. An amount of lecithin, supposedly appropriate, is added, but found to be excessive, as indicated by the undersensitivity of the antigen. Obviously, the simplest step is to add a small amount of cephalin to raise the sensitivity to standard requirements.

TABLE 8

THE ANTAGONISTIC EFFECT OF LECITHIN AND CEPHALIN IN THE TITRATION OF ANTIGENS

AMOUNTS IN ML. OF ANTIGEN AND SALT SOLUTION	A. UNCORRECTED ANTIGEN	B. 10 PER CENT DILUTION OF A WITH CEPHALIN SOLUTION B	C. 10 PER CENT DILUTION OF B WITH LECITHIN SOLUTION A
Oversensitive antigen			
1 + 1.1	Heavy agg.	Heavy agg.	Cloudy
1 + 1.3	Cloudy	Heavy agg.	Cloudy
1 + 1.5	Sl. cloudy	Heavy agg.	Too clear
1 + 1.7	Sl. clear	Heavy agg.	Too clear
Undersensitive antigen			
1 + 1.1	Tr. cloudy	Heavy agg.	Opalescent
1 + 1.3	Sl. too clear	Heavy agg.	Tr. clear
1 + 1.5	Too clear	Heavy agg.	Sl. clear
1 + 1.7	Sl. too clear	Heavy agg.	Sl. too clear

SUMMARY

Cephalin affects the titer and sensitivity of Kahn antigen. It increases the amount of salt solution necessary in the titer and the sensitivity with syphilitic serums. An antigen giving a titer with a small volume of salt solution, such as 1 + 0.9, and undersensitive compared to standard Kahn antigen, may be modified by cephalin to give a titer with a larger volume of salt solution, such as 1 + 1.3, and correct sensitivity. The extent to which antigen sensitivity is influenced by concentration and origin of cephalin has not yet been fully established. Undersensitive antigens corrected with cephalin were found to behave like standard antigen in the differential temperature and triple quantitative technics of the verification test. Oversensitive antigens were rendered still more oversensitive by the addition of cephalin. The antagonistic effect of cephalin and lecithin, the increase in sensitivity by the former lipid and the decrease in sensitivity by the latter, make possible the use of either lipid or a combination of both in the correction of antigen.

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INTRAOSSUEOUS INFUSIONS IN INFANTS*

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In recent years, increasing attention has been paid to the problem of parenteral infusions in infants. It appears now that there is not only a constant but a safe portal of entry in the medullary cavities of the long bones of the legs.

Tocantins⁹ discovered that fluids injected into the medullary cavity of bones in experimental animals entered the general circulation at a very rapid rate. This principle applied to human infusions proved to have wide application where intravenous infusion was not practical or possible.^{8, 10, 13} Previously, the danger of dislodgment of fat with production of fat embolism had been ruled out.^{3, 5}

Since 1941, several articles concerning intraosseous infusion have appeared in the literature^{1, 14} indicating that this manner of infusion in the hands of many workers is satisfactory, and that the incidence of complications is low.

TECHNIC

Our routine kit for intraosseous infusions is prepared and sterilized in advance, and contains the following: a Turkel trephine needle; a 2-cc. syringe; a 25-gauge needle; a 2-cc. ampule of one per cent procaine solution for local anesthesia; a circumcision drape; a 5-cc. syringe for aspirating the medullary cavity; and two or three gauze sponges. Alcohol-cleaned glass slides and a paraffin lined tube containing heparin are kept available for preparation of bone marrow smears. The hands of the operator are thoroughly washed and rinsed with alcohol, but rubber gloves are not used.

The site chosen for puncture is the upper one-third of the tibia or the lower one-third of the femur. Sternal infusion is not practical in children below the age of 3 to 5 years.¹¹ We prefer the tibia because the bone is directly beneath the skin and superficial fascia with little or no muscle tissue intervening. A point is selected approximately one fingerbreadth below the cartilaginous tibial tubercle and on the flat, superficial, anteromedial surface. This area is thoroughly cleaned with a suitable topical antiseptic. The circumcision drape is applied and a wheal is raised in the skin by injection of the local anesthetic. The subcutaneous tissues and the periosteum are then infiltrated with approximately 1 cc. of the anesthetic solution. The outer needle with stylet in place is thrust through the skin and subcutaneous tissue and brought into contact with the bone; slight pressure is exerted to set the needle point in the periosteum. The needle is directed distally from the epiphyseal line, the stylet removed, and the rotary trephine inserted. A hole is bored through the cortex by rotary motion of the trephine. When the trephine enters the medullary cavity, there

* Received for publication, October 11, 1946.

is a sudden drop in resistance. The outer needle is then pushed into the medullary cavity, using the trephine as a guide (Fig. 1).

We do not bind the infant or the infant's leg, but prefer to have the baby flat on a table with his legs hanging over the edge. The free hand then supports the tibia or femur, and is used to exert counterpressure when the large outer needle is inserted into the cavity. An assisting nurse keeps the infant quiet and comfortable. After the outer needle has entered the medullary cavity, the trephine is withdrawn and the bone marrow is aspirated. If bone marrow is not aspirated, the needle is not in proper position, the needle is not entirely through

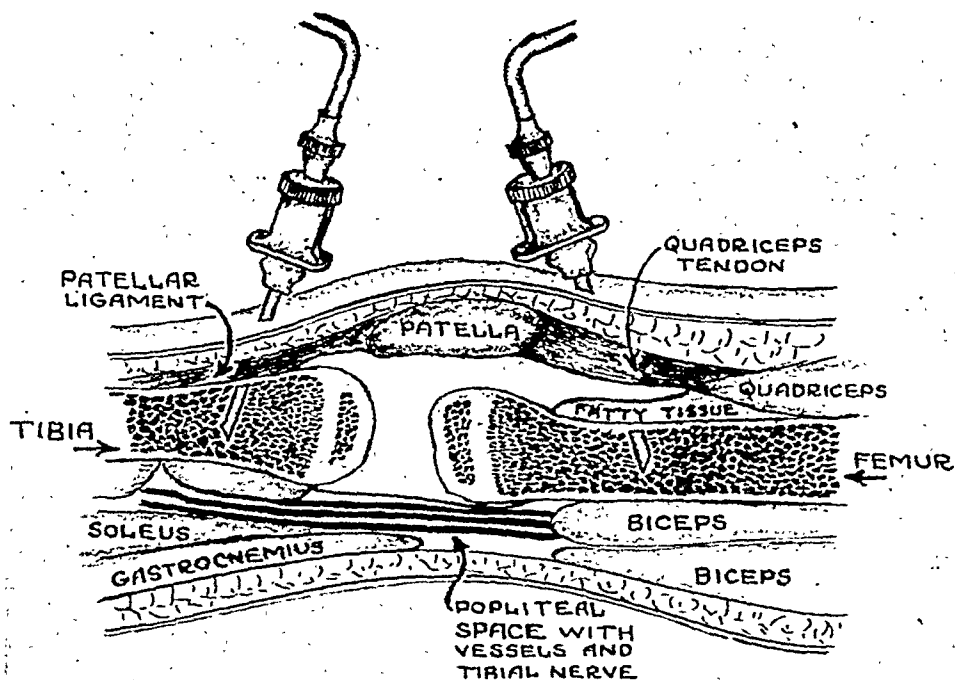


FIG. 1. DIAGRAM OF A LONGITUDINAL SECTION IN THE REGION OF THE KNEE JOINT IN A FULL-TERM, NEWBORN INFANT, SHOWING THE IMPORTANT ANATOMIC RELATIONS AND THE CORRECT POSITION OF NEEDLES FOR INFUSION

In the diagram the size of the joint cavity proper is purposely exaggerated
 Reprinted from Tocantins, L M., and O'Neill, J. F.: Complications of intraosseous therapy, *Annals of Surgery*, 122: 266-277, 1945, by permission of the J. B. Lippincott Company.

the cortex, its lumen is obstructed by a spicule of bone, the bevel is in the posterior cortex, or it has passed through both cortices. If aspiration is not successful, rotation or gentle withdrawal of the needle may correct the malposition. Infusion is not attempted until bone marrow is aspirated. When bone marrow is aspirated, the infusion is started quickly in order to prevent clotting of the aspirated marrow in the needle.

When the transfusion is completed, the needle is rotated slightly to loosen it from its seat in the cortex and then withdrawn immediately. A sterile pressure pad is placed over the puncture site and firm pressure maintained for five minutes. This allows the blood in the defect to coagulate and form a seal. A sterile

pressure dressing is applied, but care is taken not to constrict the extremity. The dressing is removed in forty-eight hours.

We have used two methods of infusing fluids: first, the direct injection with a two-way stopcock connecting syringe and needle and the infusion flask; and second, the continuous intraosseous drip. For continuous infusion, the bone offers a firm emplacement for the needle which is not easily dislodged by the infant. This constitutes one of the real advantages in using the medullary cavities for continuous infusion.

Femoral puncture necessitates thrusting the needle through the suprapatellar bursa and the quadriceps femoris muscle but, otherwise, the technic is similar to tibial puncture. The site of choice is slightly medial and about two cm. above a line drawn through the condyles. This site avoids passage of the needle through the main body of the quadriceps femoris muscle. For continuous drip infusions, we prefer to use the tibia because the needle and tubing may be attached to the leg in such a manner that the knee joint does not have to be immobilized. In femoral puncture, the needle is placed through the quadriceps tendon and may work loose with flexion and extension of the leg.

In our hands, this technic constitutes a quick and accurate method for bone marrow puncture. The rotary trephine offers the advantage of requiring little force, lessens the possibility of going through both cortices, and decreases the trauma to the periosteum and the bone. There are errors of technic which should be carefully watched for and guarded against: both cortices may be pierced; the needle may be so directed that it lodges in the cortex and does not enter the medullary cavity; the bevel of the needle may be only partly in the medullary cavity; and if the cortex is comminuted in making the tap, leakage occurs around the needle subperiosteally, or into the subcutaneous tissues (Fig. 2).

RATE OF FLOW

With direct injection of fluids, the rate of flow in 94 injections varied from 0.6 cc. to 11.0 cc. per minute (average 4.2 cc. per minute) and with continuous drip in 18 injections, the flow varied from 15 to 40 cc. per hour.

There is apparently little or no discomfort to slow infusions, but if the fluid is injected under pressure, there is definite discomfort which may be due to increased intramedullary pressure.

REVIEW OF CASES

We have given 112 bone marrow infusions in 40 infants between the ages of 12 hours and 20 months (Table 1). One of these infants weighed three pounds. Of the 40 infants, 29 are still alive and had no complications. None of the deaths was attributed either directly or indirectly to intramedullary infusions. Of the 112 attempts at intraosseous infusion, 97 were successful on the first attempt. In only one infant, despite two attempts, were we unable to use this method. In this infant, the cortex was unusually dense. Routinely, anteroposterior and lateral x-rays of the bones were taken one or two days following the last infusion and again from three to six weeks later.

In most instances, the infusion fluid was whole blood but, when indicated, we also used 5 and 10 per cent glucose in distilled water and in saline; also plasma, Hartmann's solution, ascorbic acid, Vitamin B complex, and penicillin. We have not used solutions of sulfonamides, insulin, antitoxins, or antiserums which have been given in this manner,^{1, 10} but would not hesitate to do so if the need arose. The amount and type of fluid given were determined by the need

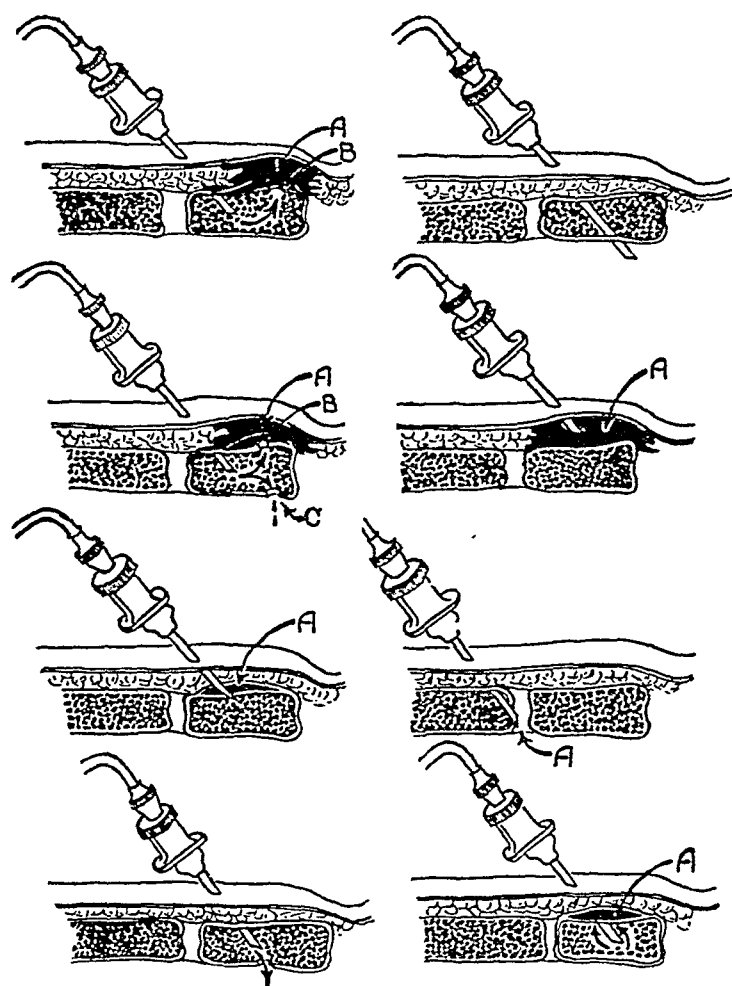


FIG. 2. COMPLICATIONS RESULTING FROM VARIOUS ERRORS IN TECHNIC OF INSERTING A NEEDLE INTO THE MARROW CAVITY

Each drawing represents a sagittal section of the manubrium and upper portion of the body of the sternum of an adult man. The same errors are possible when the long bones of the legs of infants are used. A, periosteum; B and C, sites of previous recent punctures.¹¹

of the patient. Blood infusions in newborn babies were usually given in amounts varying from 35 to 50 cc., and were repeated as often as three times a day. Older infants were given from 50 to 100 cc., depending on their size and need. One infant was given 1610 cc. of fluids during fifty-eight hours by continuous drip.

Unsatisfactory infusions have been due to several causes. There may be extravasation of blood through the defect at the site of the former puncture.

TABLE 1
 SUMMARY OF 112 INTRAVENOUS INFUSIONS IN 40 INFANTS

IN- FANT NUM- BER	CLINICAL DIAGNOSIS	METHOD OF INFUSION	FLUIDS INFUSED	TOTAL NUM- BER OF CC. IN- FUSED	NUMBER OF TAPS			COMPLICA- TIONS
					Total	Successful	Unsuccessful	
1	Erythroblastosis fetalis	Direct	Whole blood	50	1	1	0	None
2	Erythroblastosis fetalis	Direct	Whole blood	375	6	4	2	None
3	Erythroblastosis fetalis	Direct	Whole blood	190	5	5	0	None
4	Erythroblastosis fetalis	Direct	Whole blood	145	3	3	0	None
5	Erythroblastosis fetalis	Direct	Whole blood	330	5	5	0	None
6	Toxic bone marrow de- pression	Direct	Whole blood	100	2	2	0	None
7	Lymphatic leukemia	Direct	Whole blood	25	1	1	0	None
8	Bronchopneumonia	Direct	Plasma	80	1	1	0	None
9	Bronchopneumonia, inter- stitial pneumonitis	Direct	Whole blood	190	2	2	0	None
10	Hypoplasia of bile ducts, obstructive hepatitis	Direct	Whole blood	40	1	1	0	None
11	Premature, severe icterus	Direct	Whole blood	30	1	1	0	None
12	Erythroblastosis fetalis, necrosis right lobe of liver	Direct	Whole blood	58	1	1	0	None
13	Premature, intracranial hemorrhage	Direct	Whole blood	45	3	1	2	None
14	Second and first degree burns	Direct	Whole blood	370	4	3	1	None
15	General spasticity, anoxic encephalopathy	Direct	Whole blood	125	4	3	1	None
16	Diarrhea, dehydration	Direct	Plasma, 10% glucose	138	1	1	0	None
17	Diarrhea, anemia eczema	Direct	None	0	2	0	2	None
18	Bronchopneumonia	Direct	Whole blood	55	1	1	0	None
19	Erythroblastosis fetalis, melena	Direct	Whole blood	210	3	3	0	None
20	Erythroblastosis fetalis	Direct	Whole blood	235	0	5	3	None
21	Celiac disease, toxic bone marrow depression	Direct	Whole blood	455	9	8	1	None
22	Diarrhea	Direct	Whole blood	50	1	1	0	None
23	Intussusception	Direct	Whole blood	150	1	1	0	None
24	Prematurity	Direct	Whole blood— Hartmann's solution 5% glucose	600	6	6	0	None
25	Diarrhea, marasmus bron- chopneumonia lung abscess	Direct contin- uous	Whole blood, Hartmann's solution, 5 and 10% glucose, vitamins B and C	3315	7	7	0	Subcu- taneous abscess

TABLE 1—Continued

IN- FANT NUM- BER	CLINICAL DIAGNOSIS	METHOD OF INFUSION	FLUIDS INFUSED	TOTAL NUM- BER OF CC. IN- FUSED	NUMBER OF TAPS			COMPLICA- TIONS
					Total	Successful	Unsuccessful	
26	Diarrhea	Direct	Whole blood	50	1	1	0	None
27	Diarrhea	Direct	Whole blood,	990	3	3	0	None
		contin-	plasma, 5%					
		uous	glucose					
28	Diarrhea, vomiting	Direct	Whole blood,	260	4	3	0	None
		contin-	plasma,					
		uous	Hartmann's					
			solution					
29	Pneumonia, anemia (nutritional)	Direct	Whole blood,	75	1	1	0	None
			saline					
30	Diarrhea	Direct	Whole blood	40	1	1	0	None
31	Premature, vomiting	Direct	Whole blood	60	5	2	3	None
32	Erythroblastosis fetalis with general edema	Direct	Whole blood	120	2	2	0	None
33	Diarrhea	Direct	Plasma, 5%	220	2	2	0	None
		contin-	glucose					
		uous						
34	Diarrhea	Direct	Whole blood.	325	1	1	0	None
		contin-	5% glucose					
		uous						
35	Intestinal obstruction	Direct	Whole blood	50	1	1	0	None
36	Post-operative cleft palate	Direct	Whole blood	75	1	1	0	None
37	Bronchopneumonia	Direct	Whole blood,	555	2	2	0	None
		contin-	penicillin,					
		uous	vitamins					
			A and B					
38	Feeding problem, poly- avitaminosis	Direct	Whole blood,	260	3	3	0	None
			saline					
39	Bronchopneumonia mon- gloidism	Direct	Whole blood,	275	4	4	0	None
			saline					
40	Intracranial hemorrhage (due to birth?)	Direct	Whole blood,	200	3	3	0	None
			saline					
Totals.....					112	97	15	

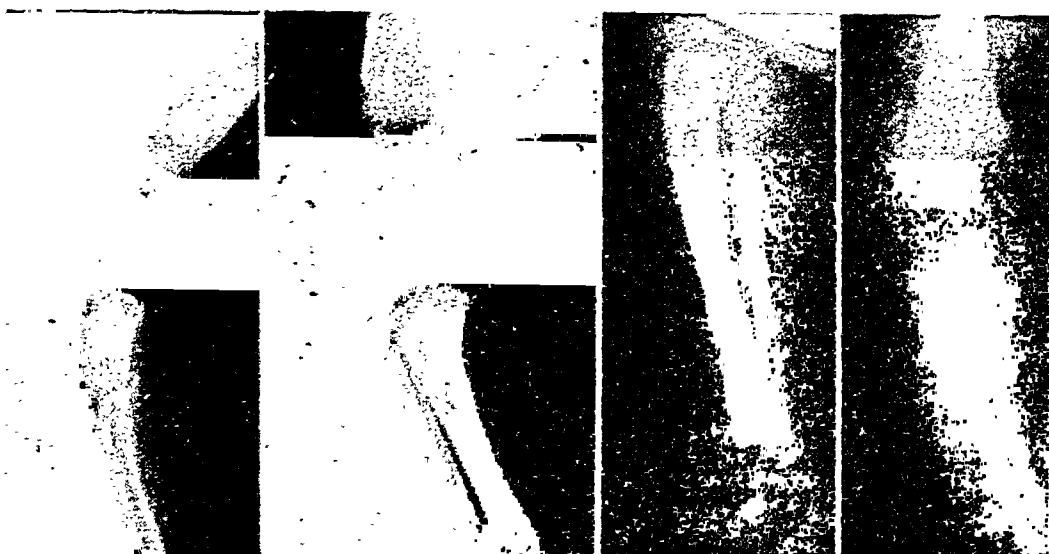
This occurs particularly when the infusion is given under pressure. Tocantins recommended that twenty-four hours be allowed to elapse between infusions in the same bone, but we have found that twenty-four hours is insufficient time. When more than one infusion must be given, we have made a routine practice of rotating the puncture sites, using first one tibia, than the other, followed by one femur, than the other. In several instances, however, we have infused into bones where the previous infusion was made less than twenty-four hours before.

If blood is forced through the site of a former puncture, the infusion should be stopped and a site in another bone selected. Other failures have occurred due to the piercing of both cortices with consequent extravasation into the soft tissues. Defective equipment, such as ill-fitting adapters on the syringes and mis-matched barrel and plunger, have complicated several infusions to the point where they were considered unsuccessful.

In the 40 infants in whom 112 bone punctures were made, we have had only one complication. A summary of this patient's case is presented.

REPORT OF CASE

A 5 week old infant was transferred from the nursery to the Department of Pediatrics, three weeks before death, because of severe diarrhea and a low grade fever, both of which



FIGS. 3a AND 3b (From left to right). X-ray films of the long bones of the legs (infant number 1, Table I) taken six days following the last infusion. The changes in the right tibia were interpreted as traumatic osteitis and periosteitis.

FIGS. 3c AND 3d. Films taken 28 days after the last infusion showed nearly complete resolution of this traumatic osteitis and periosteitis without treatment.

had not responded to the usual treatment. The infant expired on the twenty-fourth hospital day and at necropsy the following diagnoses were made: marasmus, bronchopneumonia with abscesses in lower lobe of right lung, fibrinopurulent pericarditis, chronic colitis of unknown cause, atrophic gastritis, polyavitaminosis, and subcutaneous abscesses of both thighs with no bony involvement.

During the hospital stay, seven direct or continuous intraosseous infusions were given, and 170 cc. of whole blood, 310 cc. of plasma, and 2835 cc. of other fluids (total of 3315 cc.) were thus administered.

It was the opinion at necropsy that the subcutaneous infection would probably not have occurred in a less debilitated infant and that, had this infant lived, the abscesses would have been easily managed by simple incision and drainage.

X-RAY STUDIES

In some of our x-ray studies at three days, there was a "traumatic periostitis and osteitis." These lesions were found on subsequent roentgenograms to

disappear within two or three weeks (Fig. 3). This bony lesion healed in every instance without treatment and we believe that it does not constitute a real complication. We have noticed, at the site of puncture, a firm raised nodule which seems to be attached to the bone. Arbeiter and Greengard¹ noted this also and believed that it represented bone formation at the site of puncture and could not be called a true complication.

SUMMARY

The need for, and development of, a safe and readily available route of administering fluids into the general circulation of children has been discussed. The marrow cavities of the long bones of the legs have been used with success. This method offers a rapid and easily available route of administering fluids, especially in children where the intravenous route is either impractical or impossible.

Our technic of intraosseous puncture and infusion has been described in detail. We believe that this technic allows for greater safety and fewer failures than other technics that have been described.

The incidence of complications in our series and in other series that have been reported has been very low and in no way should be considered as an objection to the proper use of this method of infusion.

A summary is given in Table 1 in which 112 intraosseous infusions were attempted in 40 infants. Of these infusions, 97 were successful on the first attempt and in only one infant were we completely unsuccessful. Our only instance of complication in this series is reported.

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EFFECT OF PENICILLIN AND ANTIANTHRAX SERUM IN EXPERIMENTAL ANTHRAX*

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The object of this study was to compare the effectiveness of antianthrax serum and penicillin, the two agents commonly used in treating anthrax. A comparison of the effective doses of these agents has not yet been reported, although both have been used in human patients. The serum has been used for many years. Penicillin has been used in recent instances, among them the series of Ellingson, Kadull, Bookwalter and Howe¹ who have reviewed the history of the treatment of this disease.

The source of the disease was a virulent culture of *Bacillus anthracis* furnished by the Division of Laboratories and Research, New York State Department of Health. It had been isolated post-mortem from the blood of a man who had died of "cellulitis" and septicemia following abrasions of the shoulder from carrying rolls of roofing material made of wool. Preliminary work showed its growth inhibited by penicillin in concentration of 40,000 Oxford units.

The experimental animals were white mice, guinea pigs and rabbits, weighing respectively about 20, 300 and 1200 gm. Forty-seven mice, 22 guinea pigs and 4 rabbits were infected by subcutaneous injection of the culture, which had been grown three hours previously in its virulent rough phase in 2 per cent dextrose and triptose broth. The mice each received 0.1 cc. of broth, the guinea pigs, 0.2 cc. and the rabbits, 1 cc. Eleven mice, 4 guinea pigs and 1 rabbit were kept as controls and the rest divided into two series. One of these two groups, containing 6 mice, 7 guinea pigs and 2 rabbits, received graded doses of penicillin intraperitoneally four hours after the injection of anthrax. The other group, composed of 20 mice, 9 guinea pigs and 1 rabbit, similarly received graded amounts of antianthrax serum four hours after injection of anthrax. The animals, treated and control, were then observed for signs of anthrax. Those dying were dissected and cultures were taken from the heart's blood and spleen. The survivors were kept until all signs of disease were gone, then killed and dissected. Cultures of these were also taken. Histologic specimens of all animals were taken and examined.

The animals dying of anthrax, controls and treated, ran a typical course. There were few symptoms until a few hours before death. Then there was stupor, dyspnea, weakness, tremors and convulsions. Locally, there was severe subcutaneous edema and a hemorrhagic and gelatinous exudate, sometimes with extension between the muscle planes. Bacilli could be seen in the local lesions and often in histologic sections of liver, spleen, lung and kidney. Smears of the fluid content of these lesions revealed gram-positive, nonsporulat-

* This work was made possible by a grant from Alexander Smith and Sons Carpet Company, Yonkers, N. Y. Received for publication, October 2, 1946.

ing, encapsulated bacilli. Material from the exudates was spread on the surface of nutrient agar and blood plates and incubated at 37 C. overnight. The cultures showed the typical soft white colonies with wreathed margins characteristic of *B. anthracis*. Saline suspensions of these cultures, tested for purity, were injected into 50 white mice in amounts of 0.2 to 0.5 cc. These mice died within two days with symptoms and lesions similar to those of the experimental animals described above and similar gram-positive bacilli were recovered from them.

The penicillin used was that available for routine hospital practice at the time, 1945, and was all from the same source.* Two different lots of antianthrax

TABLE 1
EFFECT OF PENICILLIN ON ANIMALS WITH ANTHRAX

NUMBER OF ANIMALS	DOSE PER GM. OF BODY WEIGHT	NUMBER OF ANIMALS THAT DIED, DAYS AFTER INOCULATION							SURVIVORS
		1	2	3	4	5	6	7	
	<i>Oxford Units</i>								
<i>Mice:</i>									
6 controls	0	4*	2*						
1	2.5	1†							
1	3								1†
2	5								2†
1	7.5								1†
1	2000	1†							
<i>Guinea pigs:</i>									
2 controls	0			2*					
1	2.3		1*						
2	2.7	1*							1†
2	3		1*	1†					
2	3.3								2†
2	3.7								2†
<i>Rabbits:•</i>									
1 control	0			1*					
2	3.1	1†							1†

* Animal died with signs of anthrax and *Bacillus anthracis* was recovered from it.

† *B. anthracis* was not found in cultures.

serum were used. The doses of both, per gram of body weight, were comparable with those used in treating human patients, except in one infected mouse and in its noninfected control which received a great overdose of penicillin.

The results are apparent from Tables 1 and 2. Penicillin in doses of 3 Oxford units per gm. or more protected the mice. It was toxic in a very large dose, 2000 units per gm. Slightly larger doses are required in guinea pigs and rabbits. The antiserum series showed only one survivor, a mouse receiving a relatively small dose. This is in accord with the work of Kurotchkin and Reimann,² who found it ineffective in experimental animals.

* Manufactured by Charles Pfizer and Company, Inc., 81 Maiden Lane, New York City.

TABLE 2
EFFECT OF ANTIANTHRAX SERUM ON ANIMALS WITH ANTHRAX

NUMBER OF ANIMALS	DOSE PER GM. OF BODY WEIGHT	NUMBER OF ANIMALS THAT DIED, DAYS AFTER INOCULATION							SURVIVORS
		1	2	3	4	5	6	7	
<i>Mice:</i>	cc.								
5 controls	0	4*	1*						
3	0.001	2*			1*				
5	0.015	3*						1*	
6	0.002	3*	1*					2*	
6	0.0025	6*							1†
<i>Guinea pigs:</i>									
2 controls	0		2*						
1	0.001		1*						
2	0.002		2*						
3	0.003		3*						
3	0.004		2*	1*					
<i>Rabbit:</i>									
1	0.0043		1*						

* Animal died with signs of anthrax and *Bacillus anthracis* was recovered from it.

† *B. anthracis* was not found in culture.

TABLE 3
EFFECT OF PENICILLIN AND ANTIANTHRAX SERUM ON CONTROL ANIMALS

NUMBER OF ANIMALS	DOSE PER GM. OF BODY WEIGHT	NUMBER OF ANIMALS THAT DIED, DAYS AFTER INJECTION									
		1	2	3	4	5	6	7	8	9	
Penicillin											
<i>Mouse</i> 1	2000 Oxford units	1									
Antiserum											
<i>Mice</i> 1 2	0.0015 cc. 0.0025 cc.				1 2						
<i>Guinea pigs</i> 1 1	0.003 cc. 0.004 cc.							1		1	
<i>Rabbit</i> 1	0.0044 cc.				1						

Because of this result with the antiserum, a series of controls received comparable doses of this substance alone. As shown in Table 3, it was uniformly

toxic, although the animals lived longer than those infected with anthrax. Those dying after injection of antiserum alone showed congestion of the viscera and, occasionally, abscesses of the lungs. As a check on the antiserum, a small series of animals, 1 mouse, 2 guinea pigs and 1 rabbit, each received 0.004 cc. of normal horse serum per gm. of body weight. All survived. A control of the overdose of penicillin, 2000 units per gm., in a mouse proved its toxicity.

SUMMARY AND CONCLUSIONS

Penicillin is effective in the treatment of anthrax in experimental white mice in doses of 3 Oxford units per gm. of body weight, slightly more for rabbits and guinea pigs. It is toxic in very large doses.

Antianthrax serum does not stop the course of anthrax in these animals and is in itself toxic for them.

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PRIMARY PULMONARY VASCULAR SCLEROSIS

REPORT OF CASE*

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Primary pulmonary vascular sclerosis is a disease entity of unknown cause characterized by (1) pulmonary vascular sclerosis, mainly of the small arteries, and (2) hypertrophy of the right ventricle of the heart, but not of the left.

In 1907, Mönckeberg⁶ described the first case which could be classified under this strict category. His case dealt with a 33 year old woman.

In 1935, Brenner¹ collected fifteen selected cases from the medical literature and reported a case of his own. A number of reports were not included in his summary because they showed some factor, such as chronic cardiac or pulmonary disease, capable of causing secondary pulmonary vascular sclerosis, or because no autopsy was done or the description was incomplete.

In 1941, Brill and Krygier² reported a case diagnosed during life. They included their own case with nineteen others selected from the literature and carefully analyzed the signs and symptoms. Principles of diagnosis were presented. They discussed theories concerning cause and pathogenesis with special reference to the possibility of an essential pulmonary hypertension bearing a relation to strain of the right ventricle analogous to the effect of essential systemic hypertension on the left ventricle. We refer you to this excellent paper for further details.

Our purpose is to add to Brill's and Krygier's list of cases which fall into the strict category of primary pulmonary vascular sclerosis.

REPORT OF CASE

A 20 month old white, male child was admitted to the Department of Pediatrics of the University Hospitals on May 10, 1946, with a diagnosis of nephrosis made by the referring physician.

The father, a 29 year old farmer, and the mother, a 28 year old housewife, were both in good health. The mother had had one miscarriage. In addition to the patient, there were two living children, a 5 year old son and a 3 week old daughter, both of whom were in good health. There was no history of any contact with known cases of tuberculosis. The remainder of the family history was essentially negative.

During the last few prenatal months, the mother developed nausea and vomiting. After a five hour labor, at term, she delivered spontaneously. The patient weighed eight pounds at birth. His neonatal period was uneventful.

He was breast-fed for five days and, because of an inadequate supply of milk, he was given a proprietary powder milk formula. Several weeks later, he was given a formula of diluted cow's milk. Orange juice was started at 2½ months and cod-liver oil at 3 months of age. The usual additional feedings were adequate. He did not care much for meat and ate it only occasionally. Otherwise, his diet up to the time of his illness was essentially adequate.

* Received for publication, September 6, 1946.

He had received no immunizations. Until four and one-half months before admission, he seemed to be developing as a normal child and, aside from occasional "colds", he was in good health. Four and one-half months before admission, he developed a sore throat, nasal discharge, and an afternoon axillary temperature ranging from 100 to 102 F. His mother treated him with a sulfonamide drug, one-half teaspoon twice a day for three or four days and also gave him "fever pills" and "cold tablets". The afternoon fever lasted for a week, and he lost two pounds of weight. He continued to have a nasal discharge. He began to lose interest in his playthings and surroundings. With exercise, he frequently had a dry cough. He had no episodes of cyanosis.

About three weeks before admission, he was drowsy and slept all but two or three hours a day. He showed a gain of about two pounds of weight a week and appeared to be filling out in the abdomen, face, hands and legs. He began urinating about every two hours during the day and three or four times at night. No gross hematuria was noted at any time. A physician diagnosed anemia and prescribed iron.

One week before admission, he was taken to a clinic where he was found to have a rapid heart, an enlarged liver and ascites. Anemia was suspected, but no diagnosis was made and no treatment was suggested.

Three days before admission, he had marked swelling of his face, abdomen and extremities. Two days before admission he was seen by a physician who found albumin in his urine but no anemia. On the day before admission, his generalized swelling decreased.

The examination on admission to this hospital revealed a fairly well developed male child with generalized edema. He was very irritable and uncooperative. His temperature was 99 F. rectally and the respiratory rate was 26 per minute. The radial pulse rate was 94 per minute and the blood pressure was 110 mm. Hg. systolic and 70 diastolic. His height was 32 inches, weight 25 pounds, and head circumference 48.5 cm. His head had a peculiar "square" shape. The veins on both sides of his face were somewhat prominent. His eyelids and face were edematous. Fundi were not visualized because of poor cooperation. A slight mucopurulent nasal discharge was present with slight injection of the nasopharyngeal mucosa. His tonsils were moderately enlarged and slightly injected. Both lung fields were clear. On percussion, the left border of the heart was 7 cm. to the left of the mid-sternal line, and the right border was not made out. Heart sounds were of good quality and normal rhythm. The abdomen was distended and a definite fluid wave was demonstrable. The liver edge was felt about 5 cm. below the costal margin in the right mid-clavicular line. No other organs or masses were palpable. There was pitting edema of the extremities. The anterior cervical lymph nodes were palpable but non-tender. The remainder of the examination was essentially negative.

Urine examination revealed only a trace of albumin and an acid reaction. Chemical tests for blood and sugar were negative. Microscopic examination revealed an occasional leukocyte. The volume was insufficient for a determination of specific gravity. On the second day, an Esbach quantitative analysis on available urine revealed 0.5 gm. of protein per liter. On the fifth day, it was 2.0 gm. per liter. Determinations were inaccurate inasmuch as total twenty-four hour urine specimens could not be obtained because of poor bladder habits.

Blood examination revealed a hemoglobin of 11.5 gm., erythrocyte count of 5,520,000, leukocyte count of 12,150 with 35 per cent polymorphonuclears (3 per cent band forms and 32 per cent segmented forms), 3 per cent eosinophils and 62 per cent lymphocytes. Blood Kolmer and Kline tests were negative. The blood nonprotein nitrogen was 48 mg., cholesterol 154 mg., total protein 4.9 gm., fibrinogen 0.2 gm., albumin 2.2 gm., and globulin 2.5 gm.

A tentative diagnosis of lipid nephrosis was made before the results of the blood chemical studies were reported by the laboratory. The patient was given a high protein, low fat diet. On the fifth day, he weighed 26.6 pounds and was given 30 cc. of 25 per cent sucrose intravenously. On the sixth day, he weighed 23.1 pounds.

With the accumulation of more laboratory data, the provisional diagnosis of nephrosis

was seriously questioned. Because of his low serum albumin, however, and because no cardiac disease was apparent, it was decided to give him 200 cc. of pooled plasma on the seventh day. Three hours before the transfusion, his rectal temperature was 98.4 F., heart rate 116, and the respiratory rate 32 per minute. A scalp vein was used for the transfusion. After 75 cc. of normal saline, used to start the transfusion, and 25 cc. of plasma had run in by drip, he suddenly turned cyanotic and collapsed. Death was almost instantaneous. The clinical impression of the immediate cause of death was anaphylactic shock.

Blood drawn from the patient after death was tested with the pooled plasma used and did not show hemolysis or agglutination of the erythrocytes.

AUTOPSY FINDINGS

Three hours after death a complete necropsy was performed. External examination revealed mild generalized pallor and mild cyanosis of the nail beds. The abdomen protruded somewhat and there was mild, generalized puffiness of subcutaneous tissue without pitting edema, however. The pupils were round and regular but asymmetrical, the right measuring 3 mm. and the left 4 mm. in diameter.

The primary incision revealed the peritoneal surfaces to be moist, but there was no free fluid in the peritoneal cavity. The stomach contained 150 cc. of undigested food. The stomach, the ascending and a portion of the transverse colon were distended with gas. The right leaf of the diaphragm was opposite the fourth rib and the left leaf opposite the fifth rib in the mid-clavicular lines. The pleural surfaces were moist but there was no free fluid or adhesions. The pericardial sac was tensely distended with 25 cc. of clear, serous fluid.

The brain revealed a weight of 1220 gm. (normal, 1050 gm.).⁵ It was slightly firm in consistency. Evidence of disease was not found on further gross and microscopic examinations of the brain.

The heart weighed 135 gm. (normal 56 gm.).⁵ The organ showed distention of both sides with fluid blood and postmortem clots. On the left side, dilation was well within the limits of diastole; on the right side it was excessive. It was estimated that the auricle contained 100 cc. and the ventricle 120 cc. of blood. On the anterior and lateral surfaces of the right ventricle, there were multiple petechiae and small diffuse areas of hemorrhage in the pericardium. The foramen ovale consisted of a small aperture but was functionally closed. The ductus arteriosus was closed. The tricuspid valve measured 9 cm., the pulmonic valve 4.5 cm., the mitral valve 5.5 cm., and the aortic valve 3.5 cm. in circumference. The valves showed no anomalies or evidence of disease. The endocardium was smooth throughout. The interauricular and interventricular septums showed no abnormalities. The left ventricle averaged 6 mm. and the right ventricle 3 mm. in thickness. The myocardium throughout was slightly pale, but contained no focal lesions.

Microscopic examination of the heart revealed moderate vascular engorgement, more severe in the right ventricle. There was mild subendocardial edema, again more noticeable in the right ventricle. A few lymphocytes were scattered in the endomyocardial tissue.

The lungs retained an expanded shape. The tracheobronchial tree contained

a large amount of frothy serous fluid, but there was no obstruction. The pulmonary vessels contained only fluid blood and postmortem clots. The right lung weighed 105 gm. and the left lung 95 gm. (normal 83 and 74 gm., respectively).⁵ They were generally subcrepitant and contained scattered lobules which were paler and firmer than surrounding tissue. In subpleural locations in the left upper and lower lobes, there were multiple small areas of hemorrhage measuring up to 6 mm. in diameter. The cut surfaces were moist, pale and exuded a small amount of frothy serous fluid and blood.

The microscopic examination of the lungs revealed areas of interstitial and intra-alveolar hemorrhage in sections from the left lung. No thrombi were demonstrated and the hemorrhages, like those in the pericardium, were believed to be terminal on the basis of anoxia. Venous and capillary spaces showed no appreciable congestion. Alveolar walls were thickened in the small areas of hemorrhage and of collapse. A few phagocytic mononuclear cells were present within alveolar spaces.

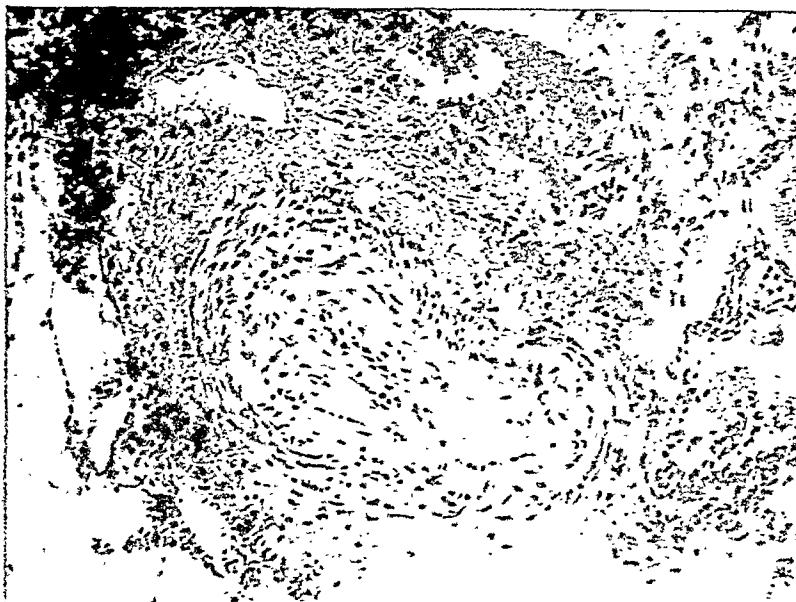
The principal finding was that of vascular sclerosis. Large arteries of the pulmonary system were not involved. Medium-sized arteries showed varying degrees of intimal hyperplasia which was often eccentric in location (Fig. 1). Some degeneration and slight hyalinization were noted in some instances.

Small arteries showed the greatest percentage of involvement, the extent of which was variable, but often severe. The lumens were extremely narrowed in the majority and completely occluded in some. In general, the adventitia showed no involvement, but in a few instances, about vessels showing the most active and disorganized hyperplasia, some adventitial hyperplasia was also evident. In some, the media was thickened due to mild hyperplasia. The intima often showed severe, regular hyperplasia (Fig. 2). In some, zones of hyperplasia were eccentric but orderly; in others, in addition to being eccentric, hyperplastic "buds" were tangled with endothelial cells. Hyperplasia of the endothelium was often seen with (Fig. 3) and without concomitant change in the intima. It frequently intruded in budlike masses occluding the lumens. This change was also noted in arterioles, venules and small veins (Fig. 4). Small arteries and their venae comitantes were occasionally seen in single hyperplastic islands (Fig. 5). In a few instances, there was associated fibrinous material in the lumens (Fig. 6) indicating partial thrombosis. MacCallum⁴ was of the opinion that thrombosis occurred as a result of the irregular endothelial hyperplasia and that recanalization followed. This was frequently noted. The prominent part played by hyperplasia of the endothelium is comparable to that in Brenner's case.¹ The elastica externa showed little change. In a few instances, the elastica interna was split, fragmented and mildly hyperplastic. In such cases, hyperplasia of the media, intimal and endothelial layers appeared intermingled and continuous.

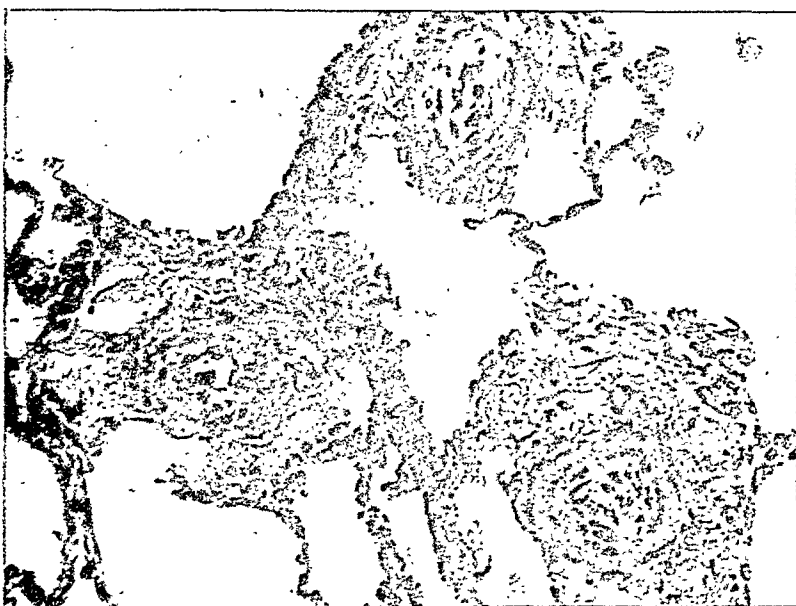
FIG. 1. Medium-sized pulmonary artery showing moderately severe, eccentric intimal proliferation and thickening. Hematoxylin and eosin stain. $\times 150$.

FIG. 2. Small pulmonary arteries showing orderly hyperplasia of the intima with marked narrowing of the lumens. Hematoxylin and eosin stain. $\times 300$.

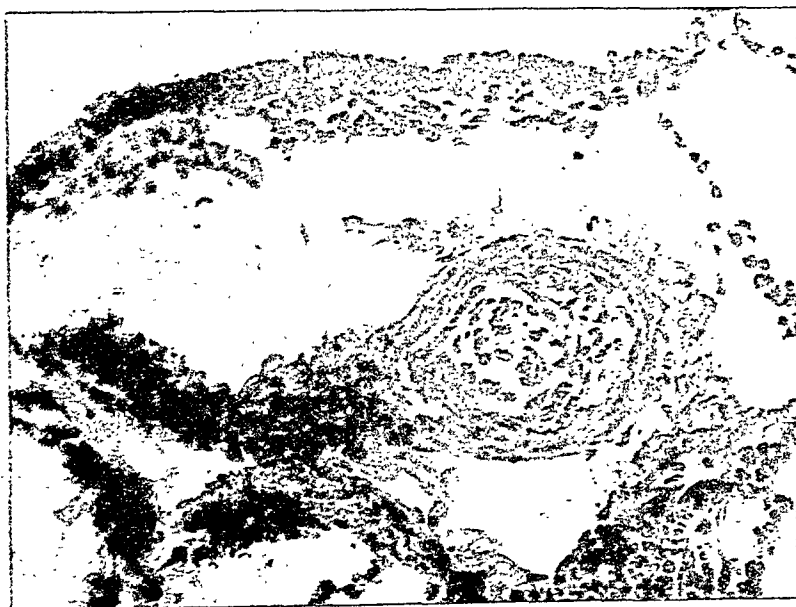
FIG. 3. Small pulmonary artery showing hyperplasia of the intima and endothelium with occlusion of the lumen. Hematoxylin and eosin stain. $\times 400$.



1



2



3

FIGS. 1-3

For the most part, there was no inflammatory infiltration. In a few instances, however, occasional lymphocytes and polymorphonuclear leukocytes were found scattered throughout the entire wall. This finding was noted particularly in vessels which lay adjacent to bronchioles containing a few inflammatory cells in the tunica propria.

Numerous vessels of all sizes escaped with no involvement whatever. Inasmuch as there was no other pulmonary disease present, no localizing factor was found.

The remaining necropsy findings consisted of passive congestion of the abdominal viscera with the organs, particularly the liver, showing an increase in weight. No other evidence of disease was found in the pituitary gland, thyroid, thymus, liver, spleen, gastrointestinal tract (except for segments of gaseous distention), pancreas, adrenals, or the genito-urinary system. The intrinsic vessels in these organs showed no arteriosclerosis. The buccal cavity, oral pharynx and larynx were not abnormal.

The culture of heart blood taken at the time of autopsy revealed no growth in sixteen days. Cultures of intestinal contents were negative for Eberthella, Salmonella and Shigella groups of organisms. Cultures of the nasopharynx revealed no beta-hemolytic Streptococci. Hemolytic *Staphylococcus aureus* and *Escherichia coli* were recovered in moderate numbers. A large amount of *Diplococcus pneumoniae* was present. The Corynebacterium organisms present were found to be nonvirulent by virulence tests.

DISCUSSION

Proliferative pulmonary vascular sclerosis with hypertrophy and dilation of the right ventricle of the heart and passive congestion of the viscera, indicative of heart failure, were the outstanding findings in this case. It is postulated that the terminal infusion of plasma, added to the load of an already decompensated right ventricle, led to sudden failure, although 30 cc. of 25 per cent sucrose had been administered two days previously.

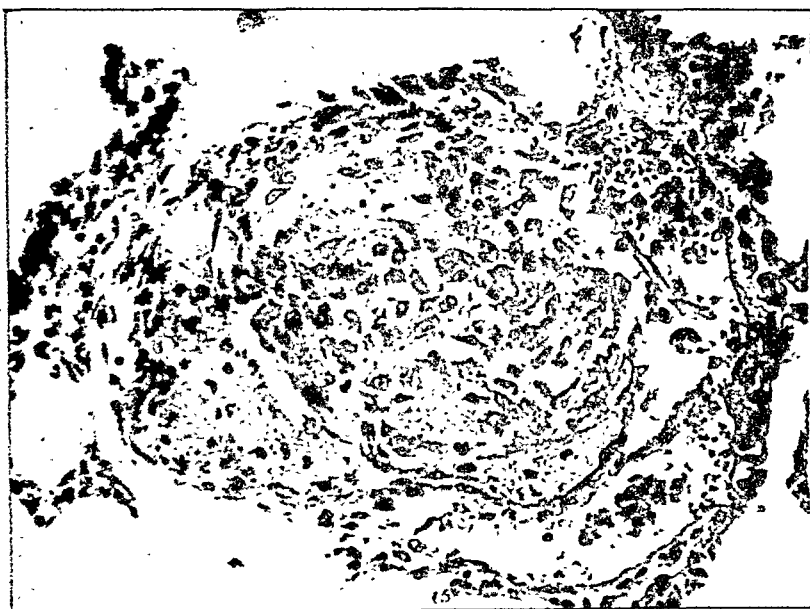
Aside from six small accessory splenic nodules at the hilus of the spleen, no congenital anomalies were found. There was no disease other than the pulmonary vascular sclerosis and those clearly connected sequelae which have been described.

Specific clinical and pathologic criteria for the diagnosis of primary pulmonary vascular sclerosis have been listed by Brill and Krygier,² Killingsworth and Gibson,³ and Brenner¹ and include: (1) cyanosis and edema which may be slight or present only at intervals; (2) clinical and electrocardiographic evidence of hypertrophy of the right ventricle of the heart but not of the left; (3) an ac-

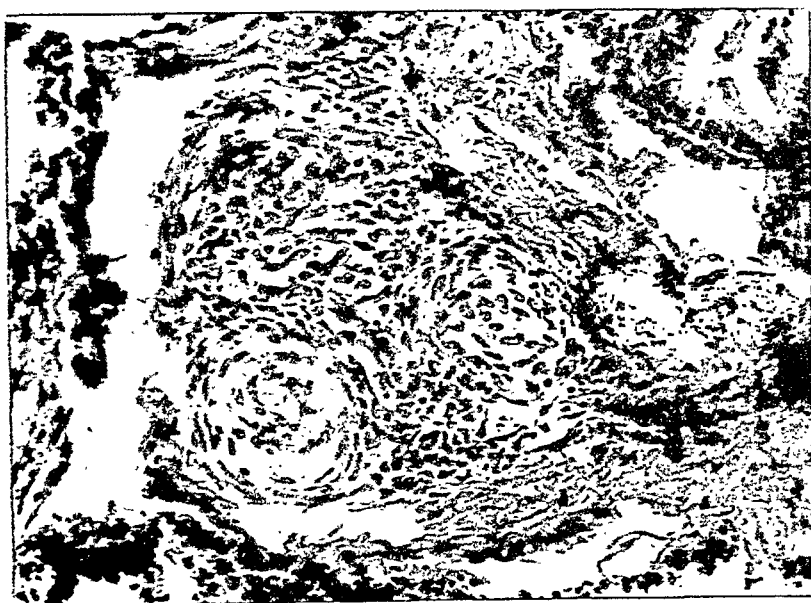
FIG. 4. Pulmonary vessel (probably a venule) showing advanced disorganized endothelial hyperplasia practically filling the lumen. Hematoxylin and eosin stain. $\times 300$.

FIG. 5. Small pulmonary arteries and vein showing intimal hyperplasia in the arteries with narrowing of the lumen in one and occlusion in the other. There is marked endothelial hyperplasia in the accompanying vein. Hematoxylin and eosin stain. $\times 300$.

FIG. 6. Pulmonary artery showing hyperplastic "buds" of endothelium with interstitial thrombosis. Hematoxylin and eosin stain. $\times 100$.



4



5



6

FIGS. 4-6

centuated pulmonic second sound but no valvular or congenital heart disease; (4) no evidence of chronic pulmonary disease; (5) x-ray evidence of enlargement of the right side of the heart; (6) no evidence of syphilis, rheumatism, tuberculosis or other disease affecting the heart or lungs.

During the period of clinical observation, the diagnoses of cardiac disease and decompensation were not specifically evident and detailed investigations were not made in the presence of suggested disease of the kidneys. We wish to point out the absence in this patient of dyspnea, orthopnea, cyanosis, fainting spells, chest pain, vomiting, dizziness, hemoptysis, and cardiac murmurs and thrills. These were relatively common signs and symptoms in the twenty cases analyzed by Brill and Krygier.² A long history of specific symptoms and signs of the disease was not present in this child 20 months of age.

The literature on the subject is well supplied with theories on cause. We are unable to make positive contributions in spite of our fairly complete post-mortem studies.

SUMMARY

A case report of a child 20 months of age with a four months' history of lassitude and a three weeks' history of polyuria and generalized edema is presented. He died during the beginning of a plasma infusion. Primary pulmonary vascular sclerosis with hypertrophy and dilation of the right side of the heart and signs of cardiac failure were found.

Careful analysis of the family and life histories and of the autopsy findings revealed no indication of a causative factor.

The absence in our patient of clear cut symptoms and signs of interval cardiac decompensation, which have been noted in other case reports involving older patients, emphasizes the need for careful clinical observation and necropsy examination to establish the diagnosis of primary pulmonary vascular sclerosis in a child of this age.

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BOOK REVIEWS

Acetanilid. A Critical Bibliographic Review. By MARTIN GROSS, M.D., Research Assistant (Assistant Professor), Laboratory of Applied Physiology, Yale University. 155 + XVII pp., 8 illus. \$3.00. New Haven: Hillhouse Press, 1946.

It is characteristic of many branches of medical science today that the literature has become so extensive and varied and the material so highly specialized that it is difficult for the general reader, or for the investigator not in the particular field, to secure an adequate picture without help. It is as aid to such readers that the volume under review and similar volumes are presented. This is the first of a series of critical reviews of the literature on analgesics and sedative drugs and is concerned with a drug introduced into medicine more than a half century ago and widely used as an antipyretic agent. Although largely replaced for this purpose by other drugs, acetanilid assumed and has continued to assume importance as an analgesic, particularly in certain proprietary medicines. Because of certain controversial aspects of the use of acetanilid, it is of particular importance to have a critical review of the literature which has accumulated since the discovery of the pharmacological properties of the compound in 1886.

More than 700 references are cited and discussed. The scope may be indicated by a survey of the section headings: history, physicochemical properties, fate of acetanilid in the body, therapeutic use, pharmacology and toxicology, acetanilid poisoning, influence of other drugs on therapeutic and toxic action of acetanilid, tolerance, and the question of addiction and habituation.

Perhaps one cannot expect that in a volume of this sort, the personal opinions of the author should be expressed. Nevertheless, it would have been helpful if the author had presented at the end of the volume a summarized statement of the present status of the drug as generally accepted by pharmacologists and therapists of today.

The format of the volume is good and the book should be of value to workers in the field. Further volumes in the series in which similar reviews of other analgesic and sedative drugs are to be presented will be awaited with interest.

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HOWARD B. LEWIS

Women in Industry. By ANNA M. BAETJER, Sc.D., Assistant Professor of Physiological Hygiene, School of Hygiene and Public Health, Johns Hopkins Hospital. 344 pp., 122 tables. \$4.00. Philadelphia and London: W. B. Saunders Company, 1946.

The author forecasts the gainful employment of sixteen million women by 1950. If this comes to pass, her book will become extremely valuable to all engaged in personnel work and to industrial physicians. Its many statements of fact and statistical reviews make it equally valuable to research workers and students. To the personnel workers and the industrial physicians, who nowadays must read as they run, or not at all, the final chapter entitled, "Summary and Conclusions" is unusually valuable. Some pertinent facts about employed women which the author successfully brings out are:

Sick-absenteeism is more frequent among women than among men, but absences are of a shorter duration. Most absences are due to ordinary illnesses and not to occupational diseases. Women seem to be no more susceptible to occupational diseases than men, and measures which are essential for the prevention of occupational diseases apply equally for men and women. On the basis of evidence available at the present, it would seem that the ability of normal women to perform mental and muscular work is not altered by either menstruation or the menopause. Pregnant women should have certain restrictions placed on their work, but with properly controlled working conditions, there is no reason why they should not work, so long as they are under the supervision of the industrial physician.

In the sections on "The Mortality and Fertility of Women in Relation to Occupation"

and the "Gynecological and Obstetrical Problems Associated with the Employment of Women," one finds topics which must be of interest to the clinical pathologist who is endeavoring to explore new opportunities in the studies of occupational health.

General Motors Corporation
Detroit

C. D. SELBY

Principles of Hematology. Ed. 3. By RUSSELL L. HADEN, M.D., M.A., Chief of the Medical Division of the Cleveland Clinic, Cleveland, Ohio. 366 pp., 171 illus. \$5.00. Philadelphia: Lea & Febiger, 1946.

The author's laudable objective is indicated as follows: "This small volume on hematology was written to simplify the studies of disorders of the blood for the student and physician." Success is attested by the appearance of the third edition, which has been revised mainly by inclusion of a few additional illustrative cases and a short description of the technic of sternal puncture.

The book contains general discussions of the hematopoietic system and blood formation, followed by detailed descriptions of technical methods. A group of chapters is devoted to elucidation of the clinical features of various hematologic disorders and the presentation of case abstracts which the student will find especially helpful, since the author's comments in explaining the blood findings, pathogenesis, and therapy of each case are clear and decisive. The 173 photomicrographs, in black and white, are generally of excellent quality. The charts and diagrams illustrating the formation, circulation and destruction of erythrocytes and leukocytes are unique in concept, simple, and easily understood.

The general tone of the book is indicated by the statement, "All disorders of the blood and variations in structure and function of the hematopoietic system are quite simple." While experienced hemopathologists heartily wish this were true, the approach is certainly one that the student will find encouraging. This attempt to "write down" to the level of the student has resulted in over-simplification, one of the book's few defects. It is regrettable that the author did not see fit to include a discussion of the Rh factor. The application of a terse and lucid style to the explanation of recent developments in this field would have been extremely useful to students and practitioners. The descriptions of the myelograms of the several conditions included in the section on bone marrow puncture are too brief to be of value as a guide for diagnosis or interpretation of the marrow lesions.

The index is adequate and there is a bibliography of 42 references. The most recent works cited appeared in 1939. The book will continue to be useful as an introduction to hematology. The clinical pathologist will find it necessary to refer to more complete texts.

Wayne University College of Medicine
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LAWRENCE BERMAN

NEWS AND NOTICES

NOMENCLATURE OF Rh BLOOD TYPES

To the Editor:

In a recent article in this Journal, a review of the present knowledge of Rh-Hr types and the latest improved nomenclature was presented (Wiener, A. S.: Recent Developments in the Knowledge of the Rh-Hr Blood Types; Tests for Rh Sensitization. *Am. J. Clin. Path.*, 16: 477-497, 1946). The nomenclature proposed was selected in order to emphasize the analogies to the scheme of the four Landsteiner blood groups. Thus, the eight Rh blood types are designated rh , Rh' , Rh'' , $Rh'Rh''$, rh_0 , Rh_1 , Rh_2 , and Rh_1Rh_2 , respectively. This designation has caused some confusion to clinicians, who sometimes mistake rh_0 for rh . For this reason, and because Rh_0 is the most important antigen clinically, and since for practical purposes in testing patients, bloods of types Rh' , Rh'' and $Rh'Rh''$ should all be considered Rh-negative, it is proposed to make a slight change in the designations, which may help further to clarify the subject for the uninitiated. Under this improved nomenclature, the three Rh blood factors are designated Rh_0 , rh' and rh'' , respectively, the capital letter being reserved exclusively for Rh_0 in order to emphasize its special position and importance. The eight Rh blood types now become: rh , rh' , rh'' , $rh'rh''$, Rh_0 , Rh_1 , Rh_2 , and Rh_1Rh_2 , respectively, while the six standard Rh allelic genes now become r , r' , r'' , R^0 , R^1 , R^2 , respectively. The new designations also have the advantage of obviating the danger of confusing the prime sign and the figure "one" in the superscripts, because the use of capitals and small letters eliminates ambiguity.

It should be emphasized that the new improvements in nomenclature do not entail any change in the oral names of the Rh types or genotypes. It is not necessary to say "large R" or "small R", as the case may be, because the qualifying subscripts and superscripts are sufficient identification. The use of capital and small letters merely acts as a visual aid in identifying the types which contain or lack the principal Rh factor, Rh_0 . The advantage of the improved names can be seen from the following grouping of the types and genes. Rh-positive bloods (Rh_0+) include types Rh_0 , Rh_1 , Rh_2 , and Rh_1Rh_2 . Rh-negative blood (Rh_0-) include types rh , rh' , rh'' , and $rh'rh''$. Gene Rh for the factor Rh_0 includes genes R^0 , R^1 , R^2 , and R^2 . Gene rh includes genes r , r' , r'' , and r^2 .

For practical purposes, one should consider that any patient who belongs to one of the four Rh-negative types is a potential candidate for Rh sensitization. For use as blood donors, on the other hand, only individuals of type rh should be selected in order to avoid reactions in patients sensitized to factors rh' or rh'' .

ALEXANDER S. WIENER, M.D.

Serological Laboratory

Office of the Chief Medical Examiner of New York City

November 7, 1946

MICHIGAN PATHOLOGICAL SOCIETY

The Michigan Pathological Society was organized in 1931 with Dr. C. V. Weller as its first president. At present there are 50 active, 24 associate and 2 honorary members. Of the nine members who were in military service, eight have been discharged and are now back in practice in Michigan.

The officers are: Dr. S. E. Gould, president; Dr. A. A. Humphrey, president-elect; Dr. D. H. Kaump, secretary-treasurer; Dr. A. L. Amolsch and Dr. S. C. Howard, councilors.

The standing committees are as follows: Nominating Committee, Committee on Ne-

crology, Program Committee, Custodian of Slides, Committee on Membership, Committee on Policy and Committee on Medical Technology.

The Society conducts meetings during the afternoon and evening of the second Saturday of February, April, June, October and December. The meetings are usually devoted to a presentation of material on a given subject, although there are occasional meetings at which material of general pathologic interest is presented. A previous report on the Michigan Pathological Society will be found in the American Journal of Clinical Pathology, Volume 15, page 488, October 1945.

The meeting places, subjects and moderators since October 1945 are as follows: October 1945, Herman Kiefer Hospital, Detroit, seminar "Diseases of the Lungs," moderator, H. C. Sweany, M.D., Chicago; December 1945, University Hospital, Ann Arbor, seminar "Diagnostic Problems in Pathology;" February 1946, Henry Ford Hospital, Detroit, seminar "Dermatologic Pathology," moderator, Hamilton Montgomery, M.D., Mayo Clinic; April 1946, Wayne County General Hospital, Eloise, seminar "Diseases of the Spleen and Lymph Nodes," moderator, Douglas Symmers, M.D., New York; June 1946, The Grace Hospital, Detroit, seminar "Diseases of the Kidneys," moderator, E. T. Bell, M.D., Minneapolis; September 1946, Providence Hospital, Detroit, in conjunction with the annual meeting of the Michigan State Medical Society, seminar "Diseases of the Breast," moderator, C. F. Geschickter, M.D., Baltimore; December 1946, University Hospital, Ann Arbor, seminar "Diagnostic Problems in Pathology."

Meetings are well attended by the members, and guests may be invited to certain meetings. Hospital residents are urged to attend as part of their regular training program.

PENNSYLVANIA ASSOCIATION OF CLINICAL PATHOLOGISTS

On Wednesday, October 9, 1946, the Pennsylvania Association of Clinical Pathologists was organized in Philadelphia.

Dr. Stanley P. Reimann of Philadelphia, President of the American Society of Clinical Pathologists called the organizational meeting to order. He discussed the purpose of the meeting and introduced Dr. T. J. Curphey, of New York, President-elect of the American Society of Clinical Pathologists, who also spoke on reasons for the formation of state associations of pathologists.

The objectives of the newly formed Pennsylvania Association are: (1) To promote closer association of its members; (2) To encourage the standardization of laboratories and to elevate the standard of work performed in various laboratories throughout the state; (3) To protect and promote the interest of the pathologist; (4) To stimulate scientific investigations and to promulgate the results of research; (5) To promote the practice of scientific medicine by wider application of clinical laboratory methods in the diagnosis and prevention of disease.

The following officers were elected: William P. Belk, Philadelphia, president; Fred O. Zillessen, Easton, vice president; and Henry F. Hunt, Danville, secretary-treasurer.

THE MINNESOTA SOCIETY OF CLINICAL PATHOLOGISTS

The Minnesota Society of Clinical Pathologists held a meeting on November 2, 1946, at the Leamington Hotel in Minneapolis. Twenty-five pathologists were present. The Society voted to establish a lectureship in honor of Dr. A. H. Sanford of Rochester, Minnesota, to be delivered at the state medical society meeting.

THE VIRGINIA SOCIETY FOR PATHOLOGY AND LABORATORY MEDICINE

The Virginia Society for Pathology and Laboratory Medicine held a meeting on October 15, 1946, at the DePaul Hotel in Norfolk. The following officers were elected: Dr. M. B. Beecroft, Newport News, president; Dr. Lawrence J. Motyca, Norfolk, vice president; Dr. M. L. Dreyfuss, Clifton Forge, secretary-treasurer; and Dr. J. H. Scherer was elected as nominee of the Society for Counsellor for the American Society of Clinical Pathologists.

THE LOUISIANA ASSOCIATION OF PATHOLOGISTS

The fall meeting of the Louisiana Association of Pathologists was held November 14, 1946, at the Marine Hospital in New Orleans. The following papers were presented: "A Rapid Method for Blood Urea Nitrogen Determination with Observations on the Use of Urease" by Dr. A. L. McQuown; "Occlusion of the Aqueduct of Sylvius" by Dr. J. B. Arcy; "Penicillin Levels in Serum and Tissue Fluid" by Dr. John C. Henthorne; "Friedländer's Bacillus Meningitis and Acute Endocarditis Superimposed on Luetic Valvulitis" by Dr. Charles L. Blumstein.

The following officers were elected: Dr. Emma S. Moss, president; Dr. Philip Pizzolato, vice president; and Dr. Charles E. Dunlap, secretary-treasurer.

ACADEMIC APPOINTMENT

Dr. J. F. A. McManus has been appointed Assistant Professor of Pathology at the Medical College of Alabama. The appointment was made in the Fall of 1946.

PERSONALS

Dr. Paul A. Van Pernis, formerly pathologist at St. Luke's Hospital, Chicago, Illinois, is now pathologist and director of laboratories at Butterworth Hospital, Grand Rapids, Michigan.

The new address of Byron W. Fraser, whose article "Microscopic Examination of Urinary Calculi" was published in the September issue of the Journal, is National Canners Association, 1739 H Street N.W., Washington, D. C., and requests for reprints can reach him there.

STUDIES IN SERUM ELECTROLYTES. XIV.

CHANGES IN BLOOD AND BODY FLUIDS IN PROLONGED FASTING*

F. WILLIAM SUNDERMAN

*From the William Pepper Laboratory of Clinical Medicine, University of
Pennsylvania, Philadelphia*

In the normal process of living the demands for nutrition are met by periodic feedings. When these are not supplied, body reserves must be utilized. As the body reserves become depleted, it is of especial interest to observe the changes that occur in the body's composition. Moreover, it is recognized that disease is closely allied to various stages of inanition and, therefore, the changes observed during fasting are useful in interpreting the changes observed in disease.

The purpose of this paper is to present the changes in the distribution and composition of the body fluids in a severely emaciated subject, whom we have reason to believe had undergone a continuous, voluntary fast for forty-five days. In so far as we are aware no systematic examinations have been made of the blood components of a human subject toward the end of such a prolonged fasting period.

Lennox, O'Connor and Bellinger² reported the changes in the nonprotein nitrogen components, sugar, bicarbonate, fibrin, cholesterol, calcium and inorganic phosphorus in the blood samples of normal and of epileptic subjects who were fasted for periods averaging fourteen days. Morgulis and Edwards²⁴ reported the changes, principally in the nonprotein nitrogen components, in the blood of five dogs that had been fasted to approximately a forty per cent loss in body weight. The few studies that have appeared in the early literature pertaining to the alterations of the blood during fasting have been reviewed by Ash.³ Among the earliest references is that of Valentin²³ who, in 1838, concluded that there was no change in the relation of blood-weight to body-weight following exhaustive starvation. As a result of the early work of Heidenhain, Panum, and Voit (referred to by Ash), it was concluded that the body is not impoverished of blood by fasting, but that, on the contrary, the organism may be plethoric. Among the most complete studies of fasting in the literature have been those undertaken by Benedict¹ on his professional faster, Levanzin, who fasted continuously for thirty-one days. Excepting hemoglobin, Benedict's studies did not include examination of other chemical constituents of the blood and serum.

The subject, Bernard, whom we have had the opportunity of studying, was a man 54 years of age, who, allegedly began his fast on March 6, 1946, one week before the Lenten season, and continued it through Good Friday. His reasons for fasting were based upon a religious asceticism in which he obtained great satisfaction from the self denial of food. By refraining from material things, he hoped to become free for the spiritual. Since 1936 he had undertaken several

* Presented at the Twenty-Fourth Annual Meeting of the American Society of Clinical Pathologists, San Francisco, California, June 28, 1946. Received for publication, November 25, 1946.

fasts, one lasting for twenty-five days in 1944, and an earlier one for nine days, during which time he maintained a complete abstinence from both food and

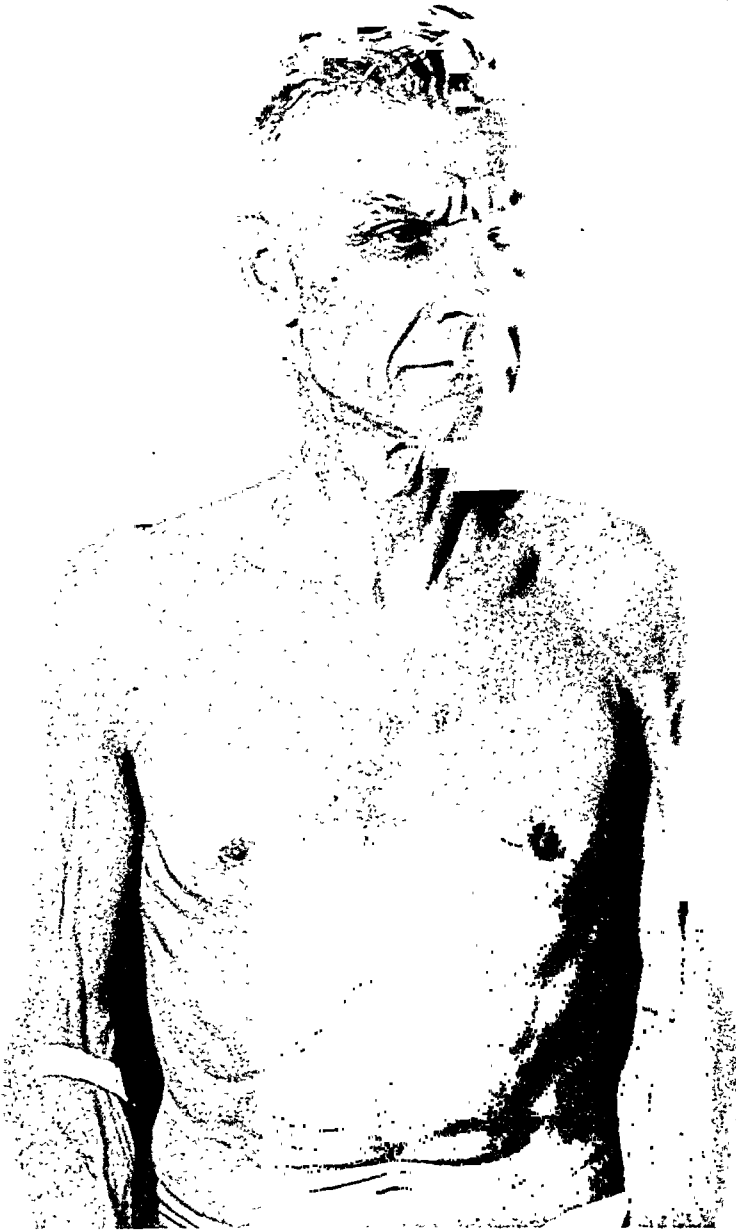


FIG. 1. PHOTOGRAPH OF BERNARD FIVE DAYS AFTER DISCONTINUING HIS FAST AND AFTER HE HAD GAINED 16 POUNDS IN WEIGHT

water. His family physician who referred him to us, had known this individual for a number of years and stated that he could be relied upon to give an exceptionally accurate, truthful account of all his actions. While a voluntary fast for

forty-five days would of itself seem to indicate an abnormal mental condition, yet in all other matters he appeared mentally normal.

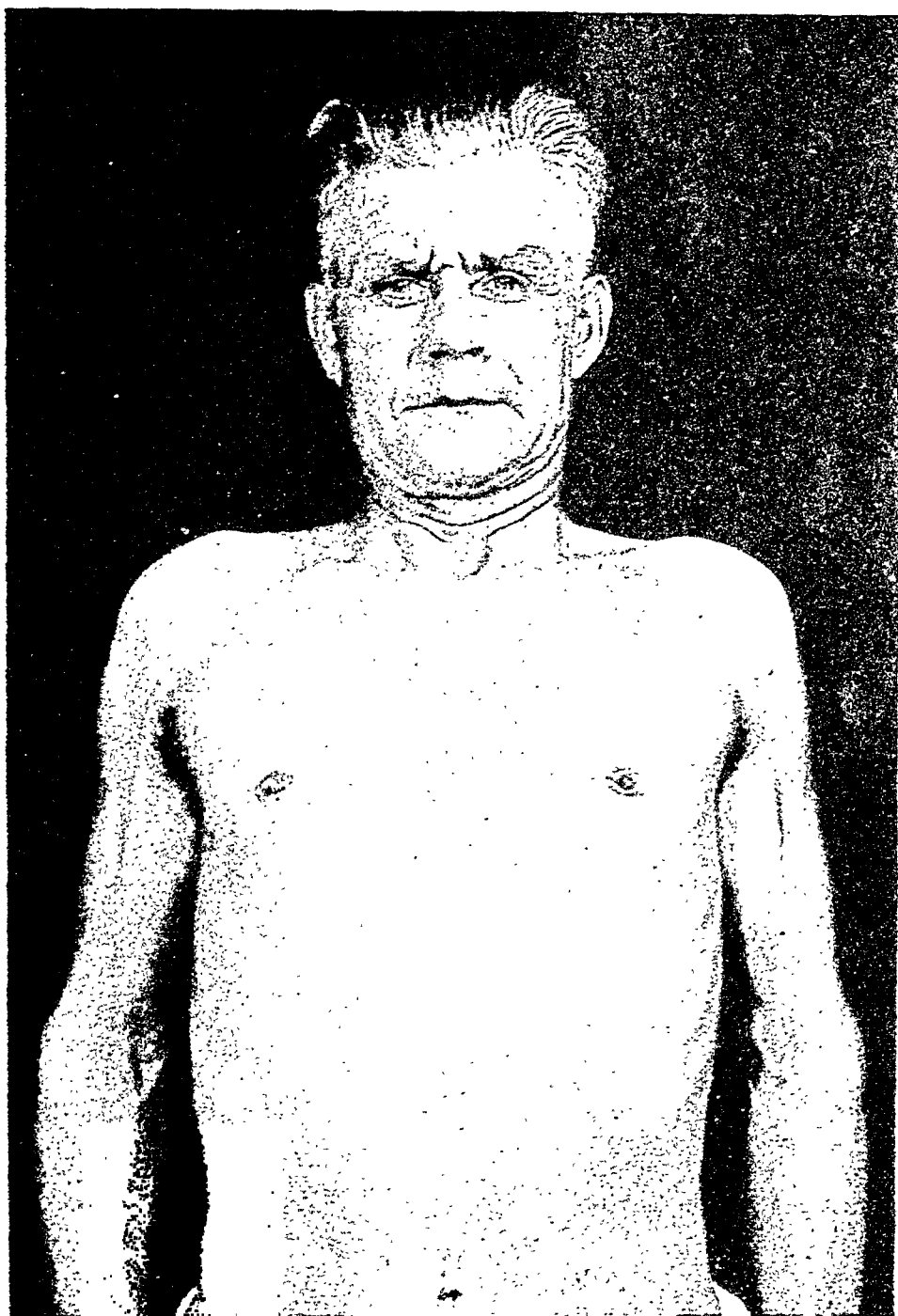


FIG. 2. PHOTOGRAPH OF BERNARD FORTY-THREE DAYS AFTER DISCONTINUING HIS FAST AND AFTER HE HAD GAINED 37 POUNDS IN WEIGHT

The subject came under our observation on the fortieth day of his fast. He was a man of medium height and gave evidence of a great loss in weight. He had a quick gait and moved about with unusual agility. In temperament he

was loquacious, sensitive, cheerful and credulous. On questioning, he appeared to be particularly anxious to be accurate in all of his statements. His memory of past events seemed to be quite good. The idea that he might undergo special examinations of scientific interest appealed to him. After some persuasion he consented to spend the last two days of his fasting period in the medical wards, *i.e.*, the forty-fourth and the forty-fifth days, the forty-fifth day being Good Friday.

On the fortieth day of his fast, Bernard's nude weight was 101 pounds. Subtracting the approximate weight of his clothing, it was estimated that at the beginning of his fast, his nude weight was 137½ pounds. At the conclusion of his

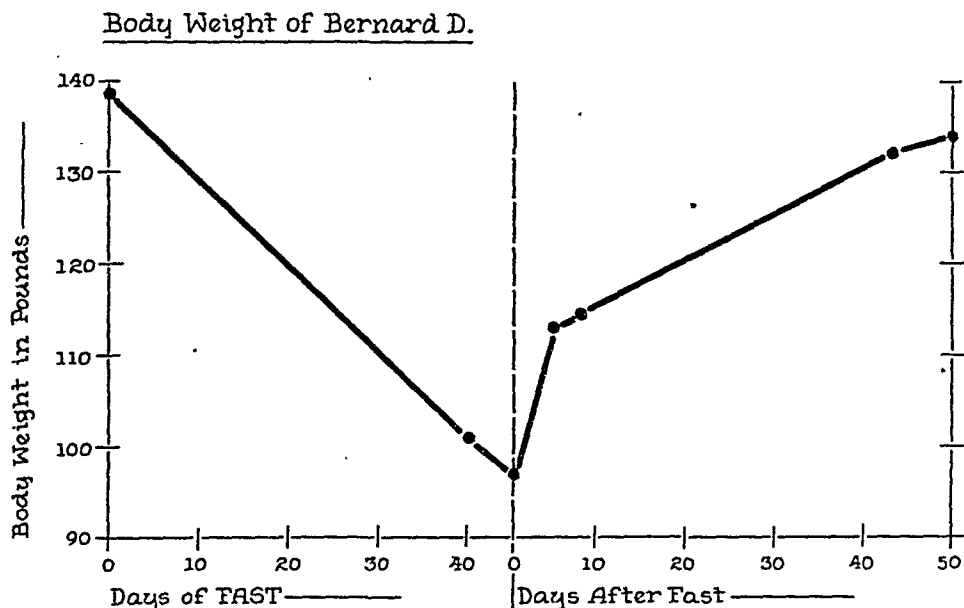


FIG. 3. BODY WEIGHT OF BERNARD.

fast, that is, on the forty-fifth day, his nude weight was 97 pounds. He had lost approximately 40½ pounds, or thirty per cent of his initial weight during this fasting period. He averred that he had taken nothing by mouth during his fasting period, with the exception of water and of a single glass of magnesium citrate, the latter having been taken because of his desire to remove all food particles from his gastrointestinal tract. He had had a small stool every second or third day which he described as "consisting of shreds resembling wet cigarette tobacco." He believed that these stools represented remnants of food in the intestinal tract and he took the magnesium citrate in order to get rid of such particles. Excepting the last week of his fast, when he was under our observation, he had lost no time from his work as a silk twister. He claimed that his feeling of hunger persisted throughout the period of his fasting state, but that it was most pronounced during the first week. From the beginning of his fast, his libido was minimal. He did not experience, at any time, numbness or tingling in his extremities or night blindness.

TABLE I
SUMMARY OF DATA ON BERNARD DURING AND AFTER FASTING

DATE	DATE IN RELATION TO DAY OF FAST	BODY WEIGHT IN LB.	SERUM COMPONENTS													URINE EXCRETION			
			Chloride mEq/L	CO ₂ volumes per cent	Inorganic Phos- phorus mg./100 ml.	Total Base mEq/L	Calcium mg./100 ml.	Magnesium mEq/L	Total Protein gm./100 ml.	Albumin gm./100 ml.	Globulin gm./100 ml.	Total Cholesterol mg./100 ml.	Esterified Choles- terol mg./100 ml.	Urea Nitrogen mg./100 ml.	Uric Acid mg./100 ml.	Creatinine mg./100 ml.	Sugar (serum) mg./100 ml.	Conadotrophins mouse units/24 hours	17-ketosteroids mg./24 hr.
April 15	41st day of fast	101																	
April 19	45th day of fast	97	74.0	100	3.4	141	12.2	2.7	6.2	5.0	1.2	198	184	24	9.0	1.4	63	—	—
April 24	5th day after fast	113	107.8	63	—	146	10.7	1.6	5.4	2.8	2.6	—	—	14	1.1	1.0	—	—	—
June 1	43rd day after fast	134	98.7	61	—	148	9.8	1.9	6.7	4.0	2.7	190	110	12	4.1	1.0	83	7.2	4.7
Normal range of values			99-104	55-60	3-4	143-148	9-11	1.9-2.0	6-7	3.3-4.3	2.2-2.8	170-190	90-114	9-17	3-4	1.0-1.2	80-110	4-16	8-15

Physical examination, excepting for evidence of severe, acute emaciation and a lowered blood pressure of 94/56 (left arm) and 90/50 (right arm), was essentially unrevealing. There was no evidence of any lesion of the tongue or mouth, or swelling of the gums, cheilosis, or skin lesions. His face had a tired expression which was accentuated by slightly drooping eyelids. The skin had lost its elasticity. The superficial veins were especially prominent and distended. His oral temperatures were decreased, averaging 97 F. Edema, or any of the usual clinical signs of avitaminosis, were not evident.

Bernard had refused to be photographed during his fasting period so that the degree of emaciation can not be shown. Figure 1 is a photograph taken five days after discontinuing his fast, at which time he weighed 113 pounds, having gained 16 pounds of his lost weight. It will be seen that his peripheral veins were still greatly distended. Forty-three days after discontinuing his fast, he weighed 134 pounds and had returned almost to his initial weight (Fig. 2). On Figure 3 are plotted the body weights during and after his fast. It will be seen that there was a sharp increase in weight within the first five days after breaking fast.

During the limited period of our observation, interest centered chiefly on the composition of his blood serum, the serum volume, the extracellular fluid volume, and the excretion of gonadotrophins and 17-ketosteroids. The results of our analyses on the serum and urine of Bernard are given in Table 1.

The following methods of analyses were used: chloride, Wilson and Ball;³⁵ carbon dioxide, Van Slyke and Neill;³⁴ inorganic phosphate, Fiske and Subbarow;¹¹ total base, Sunderman;²⁷ calcium, Clark and Collip;⁹ magnesium, Briggs;⁷ protein, including albumin and globulin, Pregl²⁵ and Kingsley;^{18, 19} cholesterol, (total and ester), Bloor⁶ and Sunderman and Razek;³⁰ urea nitrogen, Karr (modified);¹⁵ uric acid, Benedict (modified);⁵ serum volume, Sunderman and Austin²⁸ using purified congo red; extra cellular water, Crandall and Anderson;¹⁰ gonadotrophins, Levin;²³ and 17-ketosteroids, Callow (modified)⁸ and Cahan and Salter.²⁶

RESULTS

Of the inorganic components in the serum it will be seen that during fasting the concentration of *serum chloride* was strikingly decreased, while the concentration of *total base* was only slightly diminished. The decrease in the concentration of chloride was compensated by an increased concentration of *bicarbonate*. Within five days after discontinuing his fast the concentration of serum chloride had increased above the normal range of values, returning to approximately the normal range within forty-three days after the fast. The concentrations of total base and bicarbonate returned to their normal range of values within five days.

The finding of an increased concentration of bicarbonate during fasting was unexpected, since it is well known that reduction in the bicarbonate concentration attends the ketosis of fasting. In the fasted subjects studied by Lennox, O'Connor and Bellinger,²¹ the greatest reduction in bicarbonate occurred from the third to the seventh day of fasting. After the first week, these workers re-

ported that the acidosis diminished and the concentrations of plasma bicarbonate approached the prefasting levels. Measurements of the plasma pH in two of their subjects yielded only a slightly lowered value of 7.2 in one and a normal value of 7.37 in the other.

It would seem likely that the inverse relationship between serum chloride and bicarbonate concentrations observed in Bernard was probably associated with the protracted period of his fasting. It should be noted that Bernard had at no time vomited during his fasting period and that the decrease in the chloride concentration could, therefore, not be attributed to this factor. A similar decrease in serum chloride with compensatory increase in bicarbonate has been observed by Sunderman and Rose³¹ in a patient suffering from anorexia nervosa and who might be regarded as having suffered from a slow or chronic starvation for more than two years. In this patient vomiting would also appear not to be a factor in maintaining a low concentration of chloride. It should be mentioned that the diminutions in the concentrations of serum chloride in both of these patients were much greater than the diminutions in serum total base. From our studies, it would appear that the factors regulating the serum electrolytes during prolonged fasting are more directly concerned with the maintenance of a constancy in the concentration of the cations than with the distribution of the individual anions.

At the end of his fasting period, the concentrations of *calcium* and *magnesium* in Bernard's serum were considerably increased above the normal range of values. Five days after discontinuing his fast, the serum calcium returned to the normal range and serum magnesium became subnormal, returning to the normal range within forty-three days afterwards.

A similar increase in the concentration of serum magnesium has been observed in the patient with anorexia nervosa reported by Sunderman and Rose.³¹ In so far as we are aware, no other measurements of magnesium have been reported during fasting. Increased concentrations of serum magnesium are observed in individuals with hypothalamic lesions,²⁹ having subnormal temperatures and depressed metabolic states. In this connection, it might be mentioned that hypermagnesemia is a characteristic finding in the serum of hibernating animals during hibernation.³²

In five patients in whom measurements of serum calcium were made during fasting, Lennox and his associates²¹ obtained variable values, which, in general, were increased during the fasting state. On the other hand, Gamble, Ross, and Tisdall¹² found no change in the concentration of serum calcium in a child at the end of a four day fast. Although the reason for the increase in concentrations of both serum calcium and magnesium is not evident in Bernard's case, nevertheless, attention might be called to the observation that there is loss in skeletal weight during fasting and presumably release of mineral components of bone. It would seem probable that the elevated serum levels of these components might be a reflection of such skeletal changes.

The concentration of *serum proteins* at the end of Bernard's fasting period was within the normal range of values, although it will be seen that the *albumin*

concentration had increased at the expense of the *globulin*. Five days after breaking fast, the concentration of total proteins had decreased to 5.4 grams per 100 ml., owing chiefly to a decrease in the albumin fraction, the concentration of globulin having returned to normal. Within six weeks after discontinuing his fast, the concentration of both protein fractions had returned to normal.

Several days after he had resumed a normal diet, Bernard complained that he experienced "tightness" about his knees. Clinically, no peripheral edema was demonstrable. It is perhaps significant, although the mechanism is not apparent, that on previous fasts Bernard stated that he had developed pitting edema of his legs on each occasion for the first few days after breaking fast.

It would appear that the mechanism of edema formation when associated with severe undernutrition can no longer be fully accounted for on the theory of reduction in the serum colloidal osmotic pressure, since it has been shown during World War II that the appearance and severity of famine edema can not be correlated with the degree of hypoproteinemia.¹⁷ It is felt that other factors, such as diminished venous pressure and diminished elasticity and tension of the skin and subcutaneous tissues, deserve consideration and further study in the production of this type of edema. Certainly, starvation, *per se*, does not appear to produce it.

Although the concentrations of the *total cholesterol* at the conclusion of the fast and forty-three days afterwards were practically normal, it should be especially noted that the partition of *esterified* and *free cholesterol* was markedly changed, the esterified portion being greatly increased at the conclusion of the fast. In shorter fasting periods from two to seven days duration, Kartin, Mann, Winkler, and Peters¹⁶ observed only slight changes in the cholesterol distribution. We believe that the changes observed in Bernard are probably referable to the prolonged period of fasting.

Since both serum albumin and cholesterol esters are probably synthesized by the liver, the increased levels of these components at the end of Bernard's fasting period, suggest an unusual degree of hepatic activity in which these components are liberated in time of need. That this would seem to be a reasonable hypothesis is suggested by the studies of Addis, Poo and Lew^{1,2} who found after a seven day fast that the livers of rats may lose as much as forty per cent of their original protein.

The analyses of the *nonprotein nitrogen* components in Bernard's serum included *urea nitrogen*, *uric acid*, and *creatinine*. All of these were increased during the fasting period. Similar increases have been observed during fasting by Lennox, O'Connor, and Wright,²² Morgulis and Edwards,²⁴ and Hoeffel and Moriarity.¹³

Changes in the concentrations of blood urea nitrogen and urea clearance values in two dogs fasted from nine to ten days were noted by Holman.¹⁴ In both animals the concentrations of urea nitrogen were increased at the end of the fasting period and the values of urea clearance became decreased to about 10 per cent of the control values. On the first day after feeding was resumed, the concentrations of urea nitrogen in the blood became decreased and the urea

clearance values returned to prefasting levels. The increased urea nitrogen in the blood during fasting has been attributed by Morgulis and Edwards²⁴ to be the result of an accelerated catabolism of protein or an impaired excretion of nitrogenous products, or both. Since in their observations the nonprotein nitrogen values returned rapidly to prefasting levels, it would seem likely that if the elevations were due to impairment of excretion, the disability must have been merely functional in character.

Lennox²⁰ reported marked increases in the concentration of uric acid in the blood of fasted patients. In his patients the uric acid rose from an average prefasting level of 4 mg. per 100 ml. to a fasting peak of 10.6 mg. per 100 ml. of blood. Benedict⁴ has shown that the daily excretion of uric acid in the urine is reduced in starvation. After two or three days the excretion falls to about one-half of the usual endogenous level; however, after five to ten days the excretion rises to the ordinary endogenous level. Lennox²⁰ has shown that the decreased urinary output of uric acid during fasting is accompanied by a retention of uric acid in the body and an increased concentration in the blood. In children, Hoeffel and Moriarity¹³ noted that the increase in uric acid concentration appeared from the second to the fourth day of fasting and was maintained at a high level until the fast ended. The mechanism causing uric acid retention has never been satisfactorily explained.

The serum creatinine was slightly increased at the end of Bernard's fast, returning within five days afterward to the normal range of values. Of the nonprotein nitrogen components measured, creatinine would appear to be the least affected by fasting. This finding is in agreement with that reported by Morgulis and Edwards²⁴ in fasted dogs.

No gonadotrophins were detected in the urine obtained during the last day of Bernard's fast. Unfortunately, no measurements of 17-ketosteroids were obtained during the fasting period. Six weeks after ending the fast, however, the amount of 17-ketosteroids in the urine was considerably less than normal, although the gonadotrophins were excreted in normal quantities. Similar assays, in so far as we are aware, have not been reported in *fasted subjects* although, it might be noted that reduction in the urinary excretion of gonadotrophins and 17-ketosteroids, as well as estrogens, have been observed by Sunderman and Rose⁵¹ in a patient suffering from advanced anorexia nervosa. In this patient the diminution in the excretion of hormones was attributed to partial failure of pituitary, adrenal and ovarian activity.

The values for the *serum volume* and *extracellular fluid volume* are given in Table 2. It will be seen that on the last day of Bernard's fast, the serum volume was greatly increased if calculated in relation to his actual weight; when calculated in relation to his initial (normal) weight, the serum volume was only slightly diminished. Six weeks after concluding the fast, the serum volume was normal in relation to his actual weight. The calculated amount of total circulating serum at this time was only 9 per cent more than that obtained during fasting, although during this same interval, the body weight had increased more than 38 per cent.

Ballistocardiographic studies undertaken by Dr. I. Starr during the fasting

period indicated that the circulation ratings were excessive when estimated in terms of actual weight, but were normal in terms of ideal weight.

The measurements of serum volume, the ballistocardiographic values, and the prominence and distention of Bernard's superficial veins during fasting would all suggest that the volume of Bernard's circulatory bed had not been greatly altered by fasting. In this connection it might be mentioned that, excepting the brain, the heart loses percentilely less weight during starvation than other organs.

As in the estimations of serum volume, the extracellular water, or thiocyanate space, was elevated in relation to Bernard's *fasting weight*, but when calculated in relation to his normal weight it was within the normal range of values. Keys and his associates¹⁷ have recently reported an increase in the extracellular fluids,

TABLE 2
DISTRIBUTION OF BODY FLUIDS

	SERUM VOLUME			EXTRACELLULAR WATER		
	ml./kilogram of body weight		Total volume in ml.	Per cent of body weight		Total volume kilograms
	(actual weight)	(normal weight)		(actual weight)	(normal weight)	
45th day of fast.....	57.5	37.8	2539	33.4	23.6	14.7
43 days after fast.....	47.0	—	2867	24.0	—	14.6
Normal values.....		43-48			20-25	

similar to the increase observed in Bernard, in civilian volunteers who subsisted on a European type of famine diet for six months.

COMMENT

In the study of Bernard the primary importance, we believe, pertains to the data obtained on a severely emaciated individual who had obviously undergone a rigorous fast and that the actual length of his fasting period was of somewhat secondary importance. We were impressed, however, by the apparent sincerity of Bernard's claim that his fast had been continuous for forty-five days. He was a religious zealot who, in all of our contacts, took pains in being accurate in his statements. The character of the changes in his body weight during our periods of observation as well as the statements of his family physician and members of his household, lent support to his claim.

The length of time that an individual may survive without food will of course depend to a considerable extent upon his physical condition and body reserves at the beginning of his fast. Most authorities indicate that it would probably not exceed nine or ten weeks. One of the longest recorded fasts is that of Terence MacSwinney, Mayor of Cork, who after his arrest during the Irish troubles in 1920, went on a hunger strike which lasted 74 days, and terminated in his death.

SUMMARY

Studies are reported on the changes that occurred in the blood and body fluids of a severely, emaciated individual who had undergone a rigorous, voluntary fast for an alleged period of forty-five days.

It has been shown that in fasting, when the body is deprived of salt, the chloride concentration may be decreased to a much greater extent than the total base concentration. Moreover, the chloride concentration may be compensated almost entirely by the substitution of bicarbonate derived from endogenous sources. These findings direct attention to the mobility and secondary position of chloride and bicarbonate in the electrolyte pattern of serum. The factors regulating the serum electrolytes appear to be more directly concerned with the maintenance of a constancy in the cation concentration than with the distribution of the individual anions. In prolonged fasting, serum bicarbonate may replace serum chloride without any apparent detriment to the organism.

The following additional changes in the composition of the serum were observed at the end of the fasting period:

The concentrations of serum magnesium and calcium were increased.

The concentration of serum protein was within normal range of values, although the ratio of albumin to globulin was increased to 4.2.

The concentration of total cholesterol was essentially normal; the esterified portion, however, was greatly increased and represented about 95 per cent of the total cholesterol.

The concentrations of serum urea nitrogen, uric acid, and creatinine were all increased above their post-fasting levels.

No gonadotrophins were detected in the urine at the end of the fasting period.

The serum volume and the extracellular fluid volume were both elevated in relation to the fasted weight, but were essentially within the normal range of values when calculated in relation to the normal weight. The data suggests that the circulatory bed had not been greatly altered by fasting.

Within six weeks after discontinuing the fast, the subject had regained his normal weight and the concentrations of the various serum components that had been measured, were within the normal range of values.

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LYMPHOBLASTOMATOUS INVOLVEMENT OF NONLYMPHOID ORGANS*

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Under the classification of lymphoblastomas or malignant lymphomas are to be found (1) lymphatic leukemia, (2) Hodgkin's disease, (3) lymphosarcoma, and (4) reticulo-endothelioma. These diseases are found primarily in the hemopoietic organs: bone marrow, lymph nodes and spleen. Secondly, any tissue of the body may become involved with any one of these disease processes. It is the purpose of this study to investigate, from tissue removed surgically, the involvement of nonhemopoietic organs, or more specifically, nonlymphoid organs.

Bell¹ classified lymphatic leukemia and Hodgkin's disease with neoplasms because of a closer relationship to neoplastic processes than to inflammatory processes. There is no doubt, according to Boyd,² that lymphosarcoma is a true neoplasm but a sharp line cannot be drawn between lymphosarcoma and lymphatic leukemia. The cells involved in both conditions are the small lymphocytes which may be found in the various tissues of the body as metastatic lesions or infiltrations. Studies of the peripheral blood may not reveal the presence of leukemia, for one may be dealing with aleukemic leukemia or, as was found in one of the cases to be presented in this paper, the blood picture may not reveal the presence of lymphatic leukemia until the terminal stage of the disease. During remissions and in the acute forms, the leukocyte count may be normal and immature cells may be absent. According to Boyd, in lymphosarcoma the mature lymphocytes are replaced by larger, hyperchromatic cells with a small amount of basophilic cytoplasm and a round or oval nucleus with a fairly prominent nucleolus; mitosis may be present. The uniformity of cell types is a characteristic feature. The whole clinical picture may have to be taken into consideration before a distinction can be drawn between lymphatic leukemia and lymphosarcoma.

One of the characteristic features of Hodgkin's disease is its pleomorphic cell picture. There may be mononuclear and multinuclear giant cells, lymphocytes, plasma cells, polymorphonuclear cells and eosinophils. Necrosis is frequently seen and there is a marked increase of the reticulum. According to Boyd, in the occasional form known as Hodgkin's sarcoma, the uniform cellular picture of neoplasia is present. The cells are of large and uniform size, each with abundant cytoplasm and a large prominent nucleolus. Reticulum formation is not a marked feature. In the sarcomatous form there is more invasion than in the granulomatous form with its characteristic pleomorphic picture. Therefore, it may become difficult without the clinical picture to distinguish the metastatic lesions of Hodgkin's sarcoma from the other lymphoblastomas.

* Received for publication, November 27, 1946.

According to Boyd, lymphosarcoma arises in one group of lymph nodes and spreads to other groups of nodes, apparently by way of lymphatics. Later it may set up true metastasis in other organs, such as the liver, kidneys and bone marrow, the tumor cells being carried to their secondary site by way of the blood stream. Ewing⁶ stated that the kidneys are favorite sites for metastatic lymphosarcoma. The metastatic lesions may take the form of minute foci, of diffuse infiltrations, or of bulky masses of tumor tissue, composed of atypical lymphocytes with hyperchromatic nuclei. The medical literature contains relatively few reports of such cases. Michael¹⁴ reported a case of lymphosarcoma of a kidney removed surgically in which almost the entire kidney was replaced by lymphoid cells, large primary and secondary follicles with rapidly growing cells and numerous mitotic figures. Michael also stated that in his necropsy files there was not a single case of lymphosarcoma with renal involvement.

There has been considerable controversy on the subject of lymphosarcoma of the prostate. Textbooks do not mention the prostate as a site for metastatic lymphosarcoma. Ewing stated that true lymphosarcoma of the prostate is very rare and that the structure of the gland does not favor the occurrence of such tumors. Symmers¹⁸ classified the prostate as an auxiliary lymphoid focus which may be dormant. Fukase,⁷ in examining 222 prostates, found rudimentary nodes undergoing hyperplasia in inflammatory conditions. Randall and Hughes,¹⁵ as well as Tenenbaum,¹⁹ denied the existence of lymphoid tissue in the prostate, and Stirling and Ash¹⁷ doubted that primary lymphosarcoma of the prostate exists. Tenenbaum, in a review of the literature, found ten cases of lymphosarcoma of the prostate, five of which had been verified by biopsy. He reported a case of lymphosarcoma of the epididymis with metastatic foci in the prostate, mucosa of the rectosigmoid and superficial lymph nodes. Kirshbaum, Larkin and Culver,¹⁰ in a review of the literature, found fourteen cases of lymphosarcoma of the prostate and reported one of their own, bringing the total to fifteen. Out of a possible fifteen cases of lymphosarcoma of the prostate reported in the literature, only one was considered to be metastatic in origin. Ewing, in reviewing one of the reported cases of lymphosarcoma of the prostate, remarked on the possibility of a small cell carcinoma being mistaken for lymphosarcoma.

In a review of literature for the past ten years, no reference has been found to the urinary bladder as a site for metastatic lymphosarcoma. Rathbun and Wehrbein¹⁶ reported a case of lymphosarcoma of the urinary bladder in a 64 year old woman who had a ten year history of recurrent cystitis. Only five cases have been reported in the literature and in all of these cases there was a history of cystitis of several years' to twenty-five years' duration. Secondary lymph follicles were found in the bladders. Histologic examination of the tumor reported by Rathbun and Wehrbein was made by Ewing, who reported two different types of structure, one in which the tumor cells were arranged in sheets or islands resembling germinal centers and the other in which the tumor cells resembled small lymphocytes with numerous mitotic figures.

Ewing made the statement that lymphosarcoma of the testes has been a common diagnosis but he expressed the belief that in many of these cases the lesion was probably embryonal carcinoma. He has, however, studied two cases which seem to be genuine. He felt uncertain as to the exact origin of this tumor, but added that the testicle is sometimes the seat of metastatic lymphosarcoma. Dockerty and Priestley⁵ reported four cases of lymphosarcoma of the testes with the microscopic picture of infiltration rather than destruction, favoring an embolic or metastatic origin of cells.

Harrington and Miller,⁸ in reporting on lymphosarcoma of the breast, stated that three types of growth may be found: one, in which the breast is involved as part of a widespread process; another, in which the breast shows a localized lymphogranulomatous neoplasm, which later is found to be part of a systemic disease; and, a third type, localized lymphogranuloma or lymphosarcoma.

Craver and Copeland³ reviewed 164 cases in which the diagnosis of lymphosarcoma was established by biopsy or necropsy. In seventeen (10.4 per cent) of these cases, bone was involved. The bones involved in order of frequency were vertebrae, pelvic bones, skull, femur, humerus, tibia, scapula, mandible, fibula and ribs. Two types of osseous change were found, the osteolytic and osteoplastic. The involvement of the bones took place by two routes, hematogenous and direct infiltration from contiguous diseased lymph nodes.

According to Boyd, Hodgkin's disease is characterized by progressive anemia and enlargement of lymph nodes, spleen and liver. The cause is unknown and the nature of the condition is uncertain. Boyd further stated that the disease has the characteristics of an infective granuloma and a tumor. The pleomorphism of the microscopic picture suggests an inflammatory lesion, whereas its local spread and its fatal termination are characteristic of a malignant lesion. Enormous tumor-like masses may be found in the liver, spleen and kidneys. Lesions have also been found in the skin, bone and mucous membranes. Ewing mentioned that metastatic growths of considerable extent have been found in the liver or lungs and that involvement of bone marrow occurs terminally in practically all cases. Vieta, Friedell and Craver²⁰ found definite destruction of cortical bone representing an entirely different phase of osseous involvement. In eight of forty-seven cases of Hodgkin's disease in which necropsy was performed, there was destruction of cortical bone. Craver and Copeland⁴ in a group of 172 cases of Hodgkin's granuloma found twenty-seven (15.7 per cent) in which involvement of the bone was present. Two types of changes were found, osteoplastic and osteolytic. Two routes of osseous involvement were assumed, one from the marrow foci, and the other from contiguous diseased lymph nodes. Craver and Copeland found the vertebrae and pelvic bones most frequently involved and Vieta, Friedell and Craver found the vertebrae, ribs, pelvis and femora most frequently involved.

LYMPHATIC LEUKEMIA

According to Kracke,¹¹ the essential pathologic changes in chronic lymphatic leukemia are to be seen in the proliferating lymphoid tissue and the organs in

which great numbers of cells are deposited. Any organ in the body may be the seat of nodular or diffuse infiltration of lymphoid cells but the most common sites are liver, kidneys and spleen. Kracke also mentioned cell infiltration occurring in the testicle, epididymis, ovary, pancreas, esophagus, bronchi, choroid conjunctiva, nerve trunks, meninges, sweat glands, salivary glands and epicardium. McDonald and Waugh¹³ reported a case of chronic lymphatic leukemia with infiltration into the endometrium. Two cases with leukemic infiltration of the prostate producing prostatic enlargement and signs of obstruction have been reported, one by Jacobi, Panoff and Herzlich⁹ and the other by McCrea.¹²

MATERIAL AND METHODS

The material for this study was taken from patients who were admitted for surgical conditions to the Mayo Clinic from 1920 to 1945, inclusive, and from whom tissue was removed either for diagnosis or for relief of symptoms. After fixation in formalin, blocks were cut, dehydrated in varying dilutions of alcohol, cleared and embedded in paraffin. Sections 8 microns in thickness were cut and stained with hematoxylin and eosin. This paper is primarily concerned with the lymphoblastomatous involvement of nonlymphoid organs as seen in surgical material. The organs under consideration are the breast, liver, prostate, testes, uterus (endometrium), kidneys, bladder and bones. It is the purpose of this study to point out the possibilities of finding metastatic lesions in these organs.

The cases to be presented are divided into three groups. Group 1 contains seven cases in which the diagnosis was unquestionably established in the nonlymphoid organ by positive evidence of the disease process in lymphoid or hemopoietic organs. In two of the seven cases, necropsy reports were available. In all seven cases the clinical history and findings on physical examinations were very suggestive of a lymphoblastomatous process.

Group 2 contains four cases in which the clinical history and findings on physical examination were very suggestive of a lymphoblastomatous process, but tissue from a lymphoid organ was not removed. The histologic picture of the neoplastic cells in the nonlymphoid organ was so characteristic that a diagnosis was not difficult to establish.

Group 3 contains six cases in which the histologic picture of the neoplastic cells in the nonlymphoid organs gave the only positive evidence of the disease process, but the neoplastic cells so much resembled those found in group 1, in which diagnosis was unquestionably established, that it seems reasonable to assume that all cases in group 3 are those of metastatic lymphoblastoma.

REPORT OF CASES

Group 1

Case 1. The patient, a woman aged 27 years, was admitted to the Mayo Clinic in November, 1936, complaining of lumps in both breasts of a few weeks' duration. On physical examination freely movable nodules in the upper quadrants of both breasts were felt. Roentgenographic examination of the thorax revealed widening of the mediastinum suggestive of lymphosarcoma or a metastatic tumor. A wedge-shaped segment from the



mediastinal shadow. In view of the history of postmenopausal bleeding, dilatation and curettage was done and endometrial tissue was removed for histologic examination. The endometrial stroma was completely replaced by lymphocytes which were larger than normal and showed occasional mitotic figures. The endometrial glands were preserved. In view of the findings on the peripheral blood, this was unquestionably a case of leukemic infiltration into the endometrium (Fig. 3).

Case 5. The patient, a man aged 57 years, was admitted to the clinic in May, 1943, complaining of pain in the left sacro-iliac region extending down the left leg. According to the patient the pain had been present for years. On physical examination, there were palpable supraclavicular, axillary, epitrochlear and inguinal nodes. The spleen and liver were somewhat enlarged. Both testicles were hard and nodular. Studies made on stained smears of the peripheral blood were not diagnostic. Specimens from the right inguinal lymph node, left axillary lymph node and left testicle were taken for biopsy. The right inguinal lymph node was reported as inflammatory, but the left axillary node was reported as follicular cell lymphosarcoma. Histologic studies made on tissue removed from the testicle showed masses of mononuclear cells with fairly abundant faintly staining cytoplasm separated by strands of reticulum fibers. The nuclei of the cells were moderately chromatic and irregular in size and shape. Mitotic figures were fairly numerous and testicular tubules were atrophic (Fig. 4). This cell picture is consistent with that of a lymphosarcoma and it will be noted that generalized lymphadenopathy was found on physical examination and a lymph node fairly remote from the region of the testicle was involved by the tumor. In this case it is assumed that the primary tumor involved the lymph nodes and that the testis was secondarily involved.

Case 6. The patient, a man aged 79 years, was admitted to the clinic in July, 1945, complaining of dyspnea, precordial pain on exertion, anorexia, nausea, malaise, weakness and loss of weight. On physical examination, a blood pressure of 190 mm. of mercury systolic and 70 diastolic, auricular fibrillation with complete heart block, pedal edema, a mass in the right lower quadrant and a mass in the right testicle were found. A specimen was removed from the right testicle for histologic examination and diffuse masses of mononuclear cells separated by strands of reticulum were found. These cells were of medium size with fairly abundant cytoplasm and moderately chromatic nuclei of irregular shape and size. Mitotic figures were fairly numerous. The histologic picture was that of a lymphosarcoma. Two months later the patient died. A necropsy was done and the lymphosarcoma previously described was found to involve the right testicle, adductors of the left thigh, paravertebral lymph nodes, heart, left adrenal, ribs, diaphragm and intestine. Because of the extensive involvement of the organs by the neoplastic tissue, it was not possible to determine the primary site but it may be assumed that the intestine or paravertebral lymph nodes were primarily involved.

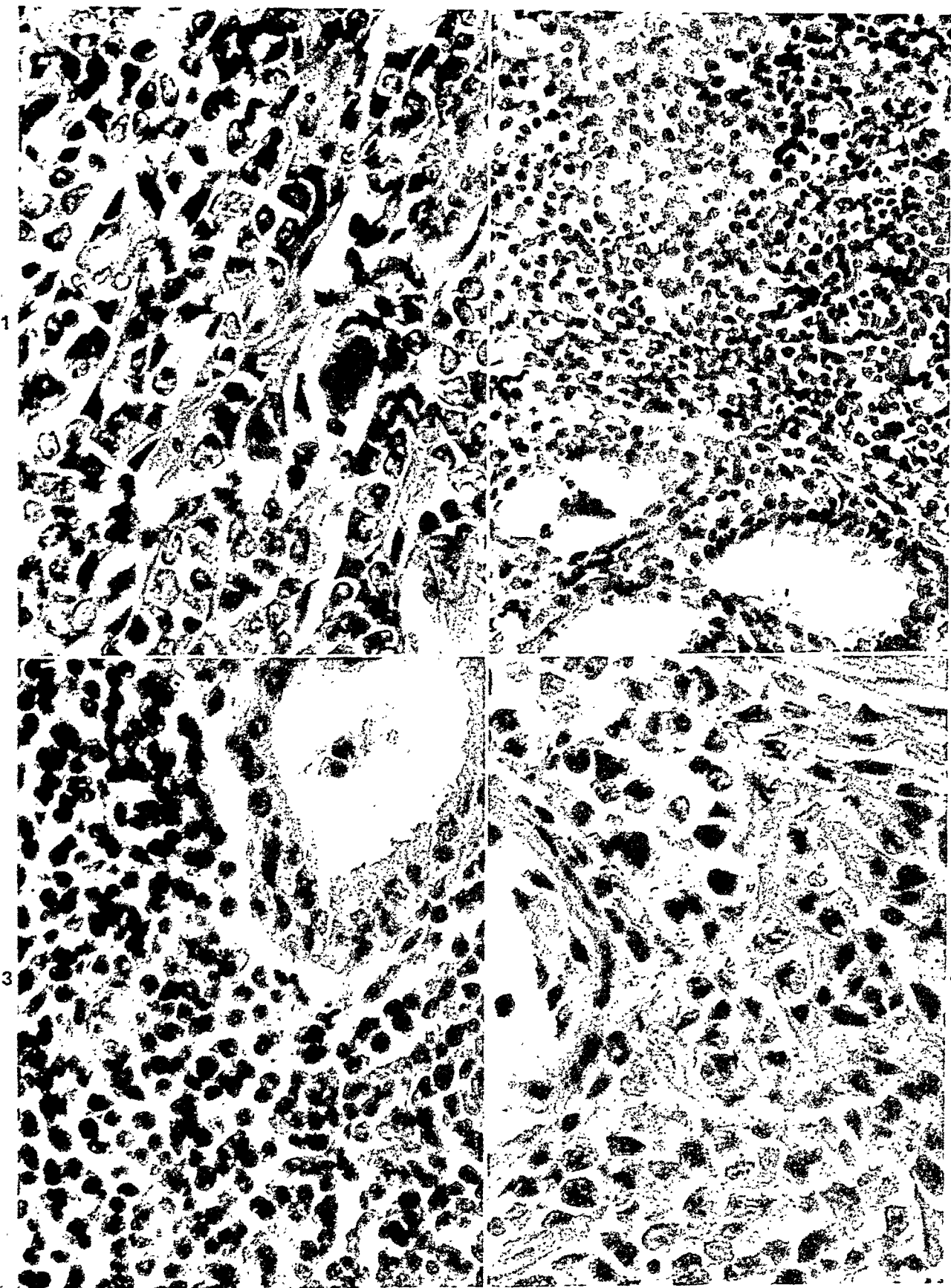
Case 7. The patient, a man aged 50 years, was admitted to the clinic in August, 1940, complaining of low back pain and loss of weight of one year's duration, and swelling of feet and ankles of three weeks' duration. Physical examination disclosed generalized peripheral lymphadenopathy. Studies made on stained smears of the peripheral blood were not diagnostic. Roentgenographic examination made on the right femur showed a destruction of a crescentic portion of bone just above the femoral epiphysis. A left cervical node and a specimen from the right femur were removed for histologic examination. Sections of the lymph node revealed a lymphosarcoma, reticulum cell type (Fig. 6a). Histologic examination of the tissue removed from the femur showed a monotonous cell picture composed of medium-sized mononuclear cells with a fair amount of pale pink cytoplasm. The nuclei of

FIG. 1. Section of breast showing neoplastic cells separated by strands of reticulum fibers. $\times 600$.

FIG. 2. Neoplastic cells with fairly abundant cytoplasm and irregular hyperchromatic nuclei scattered among prostatic glands. $\times 285$.

FIG. 3. Endometrial gland with leukemic infiltration into stroma. $\times 600$.

FIG. 4. Atrophic testicular tubule surrounded by neoplastic cells. $\times 600$.



the cells were round, oval, or irregular and many cells were hyperchromatic. Numerous mitotic figures were seen (Fig. 6b). This cell picture corresponded to that seen in the cervical lymph node and established with some certainty the metastatic nature of the bone lesion.

Group 2

Case 8. The patient, a woman aged 25 years, was admitted to the clinic in June, 1940. She stated that five years before, she had had roentgen therapy for enlarged cervical and axillary lymph nodes. No specimen had been taken for biopsy. Two years later, nodules had developed in the left breast and on the thoracic wall. Again she had been given a course of roentgen therapy and had improved as before. Three months before she came to the clinic, thoracic pain developed.

On physical examination, small palpable nodes in the right and left cervical regions, a palpable axillary node on the right side and a palpable nodule in the left breast were found. The nodule in the left breast was excised for histologic examination and revealed a pleomorphic cell picture. Numerous giant cells of the Sternberg-Reed type were scattered throughout the section. There were numerous polymorphonuclear cells resembling the neutrophils of the peripheral blood and many eosinophils and mononuclear cells with large oval or irregular-shaped nuclei, large nucleoli and fairly abundant cytoplasm. A few small mononuclear cells with scanty cytoplasm and hyperchromatic nuclei were seen throughout the section. There was an increase of the reticulum fibers with areas of fibrosis (Fig. 7a and b). This cell picture was consistent with that seen in Hodgkin's disease. The history, clinical course and response to roentgen therapy in this case along with the findings in the breast tissue, established with a fair degree of certainty the presence of Hodgkin's disease.

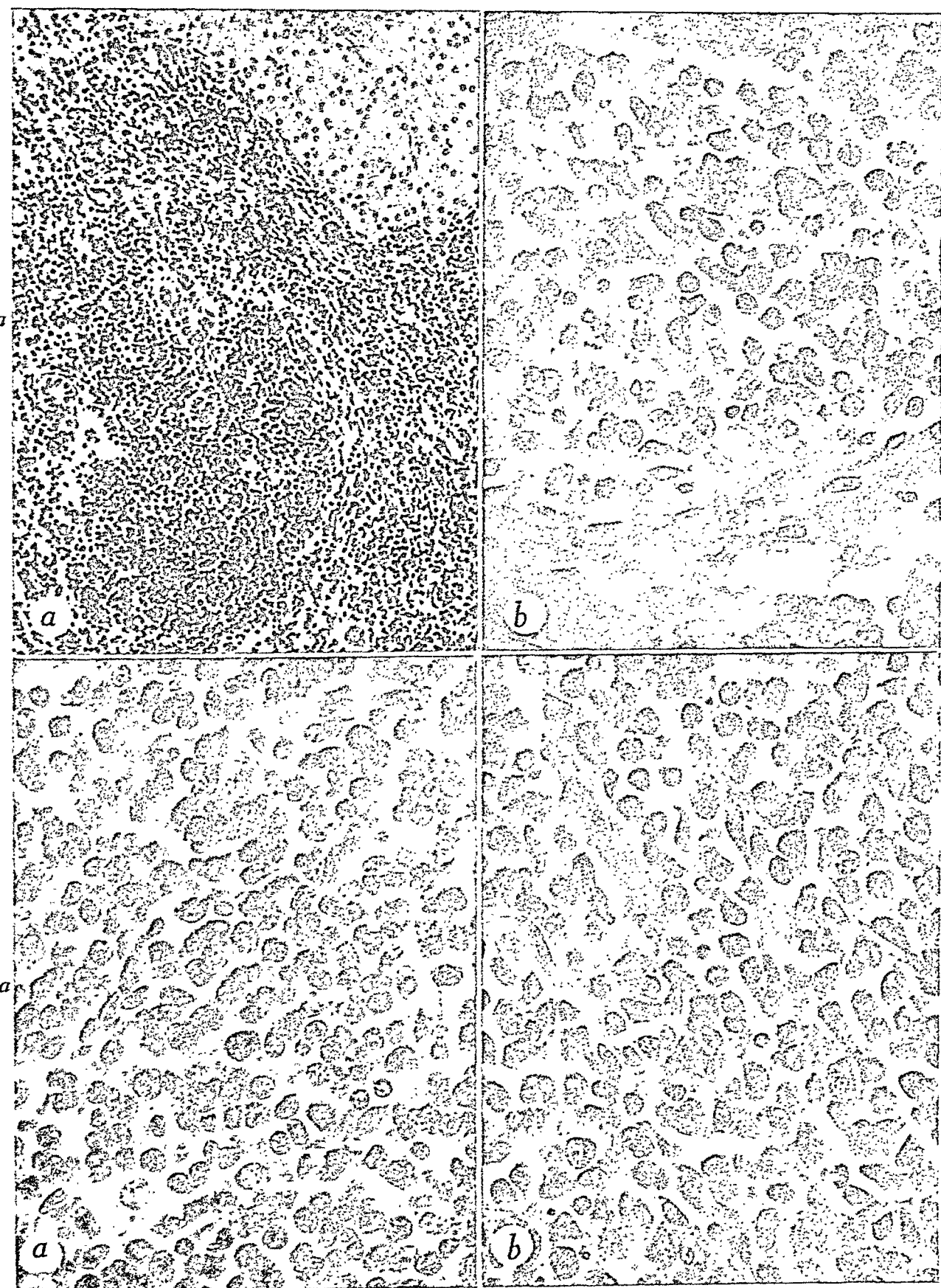
Case 9. The patient, a woman aged 43 years, was admitted to the clinic in April, 1941, complaining of loss of weight and weakness of one year's duration and cough, elevations of temperature, and a mass in the left breast of four months' duration. She stated that, four years before the present trouble developed, she had had radium treatments for carcinoma of the cervix. Soon after, there had developed a skin rash, which had been diagnosed as pellagra.

On physical examination, a mass in the left breast, and palpable liver and spleen were found. Pelvic examination gave essentially negative results. Studies made on the peripheral blood showed hypochromic anemia, but nothing diagnostic. Roentenographic examination of the thorax showed probable lymphosarcoma with infiltration of the base of the left lung and interlobar pleural thickening. Simple mastectomy was done. Histologic examination of the tissue revealed numerous mononuclear cells with fairly abundant, faintly staining cytoplasm and moderately chromatic nuclei, irregular in size and shape. An occasional mitotic figure was seen. These cells were scattered diffusely among the mammary ducts (Fig. 9). The cell picture was that of a lymphosarcoma. As additional evidence of the disease was offered by the roentgenographic examination of the thorax, it was assumed in this case that the breast was the site of metastatic lymphosarcoma.

Case 10. The patient, a man aged 57 years, was admitted to the clinic in February, 1942, complaining of nocturia, frequency, dribbling and a sense of pressure deep in the pelvis of four months' duration. On physical examination, a firm, tense, slightly enlarged prostate and palpable submental, cervical, axillary and inguinal nodes were found. A roentgenogram of the kidneys, ureters and bladder revealed a nonfunctioning right kidney. An exploratory operation was done and a retroperitoneal mass deep in the right side of the pelvis was found. A few periaortic nodes and a specimen from the right ureter were re-

FIG. 5a. Section from liver showing island of liver cord cells surrounded by masses of neoplastic cells. Note tendency of cells to form follicles. $\times 165$. b. Note mitotic figures and cells with hyperchromatic nuclei. $\times 430$.

FIG. 6a. Reticulum cell sarcoma of lymph node. $\times 600$. b. Neoplastic cells from tissue removed from femur. Note that histologic picture resembles that in Figure 6a. $\times 600$.



moved for histologic examination and were found to be of an inflammatory nature. Two weeks later, transurethral prostatic resection was done and 5 gm. of prostatic tissue was removed. Histologic examination showed, for the most part, small cells with scanty cytoplasm and hyperchromatic nuclei scattered diffusely throughout the prostatic tissue. There were a few cells with an irregular or oval nucleus and fairly abundant cytoplasm. Perineural lymphatic involvement by the neoplastic cells (Fig. 8a) and a few mitotic figures (Fig. 8b) were found. The malignant cells in this case were composed for the most part of cells resembling small lymphocytes and undoubtedly the lesion was a lymphosarcoma. The presence of peripheral lymphadenopathy was certainly suggestive of the widespread nature of the disease.

Case 11. The patient, a man aged 57 years, was admitted to the clinic in October, 1940, complaining of passing blood with the urine. This hematuria had been present for two and one-half weeks. He stated that a specimen of tissue removed from the bladder had been reported as papillary carcinoma.

On physical examination, there was generalized lymphadenopathy and an enlarged prostate. A specimen, taken from the vesical neck for histologic examination, revealed diffuse masses of mononuclear cells slightly larger than lymphocytes with rather scanty cytoplasm, hyperchromatic nuclei irregular in shape and size, and occasional mitotic figures (Fig. 10). No papillary carcinoma was seen in the sections examined. The cell picture was consistent with lymphosarcoma and some additional evidence of the disease was offered by the presence of generalized lymphadenopathy.

Group 3

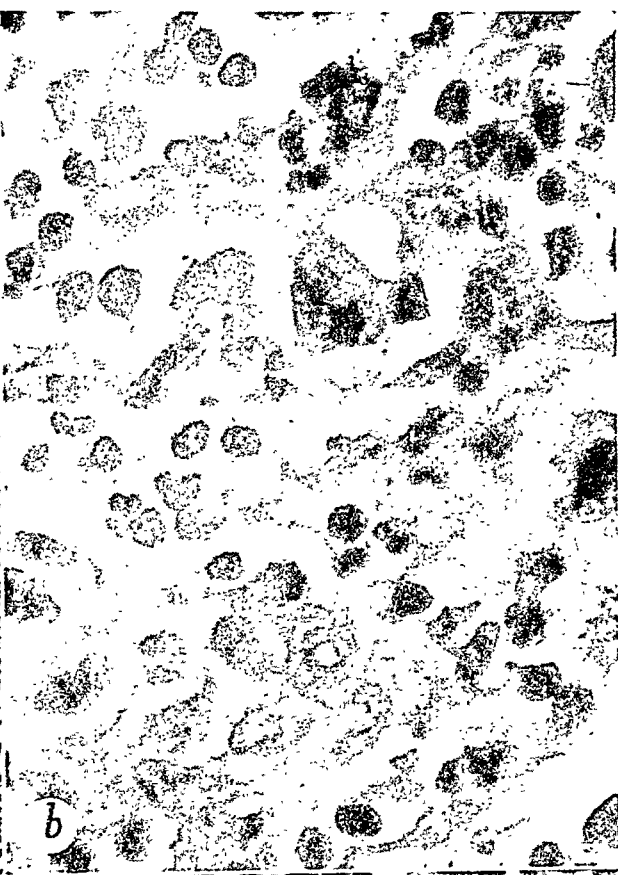
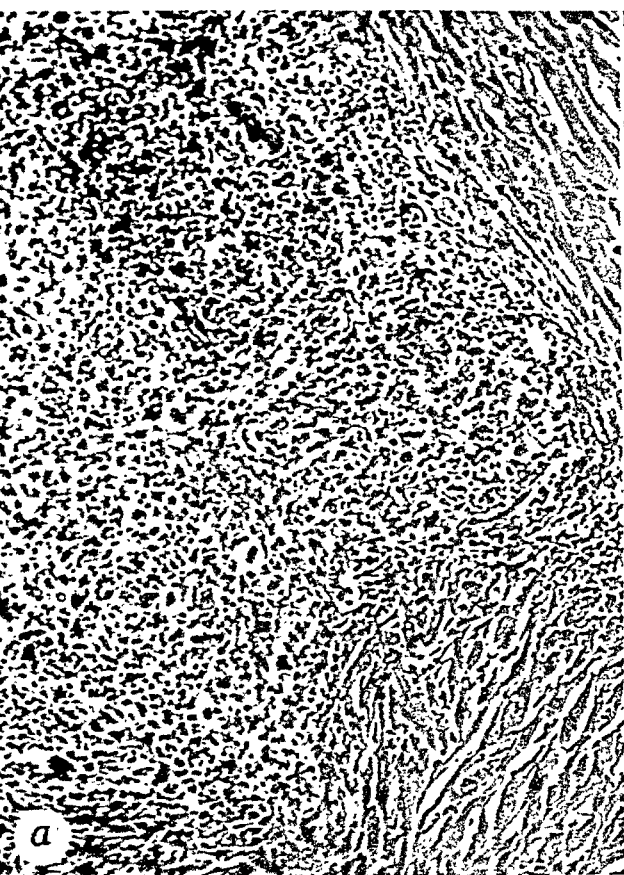
Case 12. The patient, a woman aged 28, was admitted to the clinic in December, 1920, complaining of progressive weakness during the previous year. On physical examination, palpable masses the size of walnuts, were found in both breasts. A roentgenographic examination of the thorax revealed indefinite small shadows. The right breast and axillary nodes and a specimen from the left breast were removed. On histologic examination, tissue from the right breast presented an appearance consistent with lymphosarcoma. Distributed among reticulum fibers were medium-sized mononuclear cells with fairly abundant cytoplasm and moderately chromatic nuclei, irregular in size and shape. An occasional mitotic figure was seen (Fig. 11). Histologic examination made on sections of the left breast revealed the same picture. Since the impression of the roentgenologist was that the shadows seen in the thorax were probably due to a primary tumor, it was assumed that the breast was involved secondarily.

Case 13. The patient, a man aged 72 years, was admitted to the clinic in February, 1940, complaining of frequency and burning on urination, and difficulty in starting the urinary stream with occasional hematuria of three years' duration. On physical examination, an enlarged prostate and enlarged inguinal and left cervical lymph nodes were found. Transurethral prostatic resection was done and 17 gm. of prostatic tissue was removed. Histologic examination made on the tissue revealed small round cells with fairly scanty cytoplasm and an oval or irregular-shaped nucleus. The majority of the cells, which were scattered diffusely throughout the sections of prostatic tissue examined, were hyperchromatic and an occasional mitotic figure was seen (Fig. 12). The neoplastic cells in this case resembled the small lymphocyte and this case was considered as an example of metastatic lymphosarcoma in the prostate. The enlarged lymph nodes found in the cervical region were suggestive of the presence of the disease elsewhere in the body.

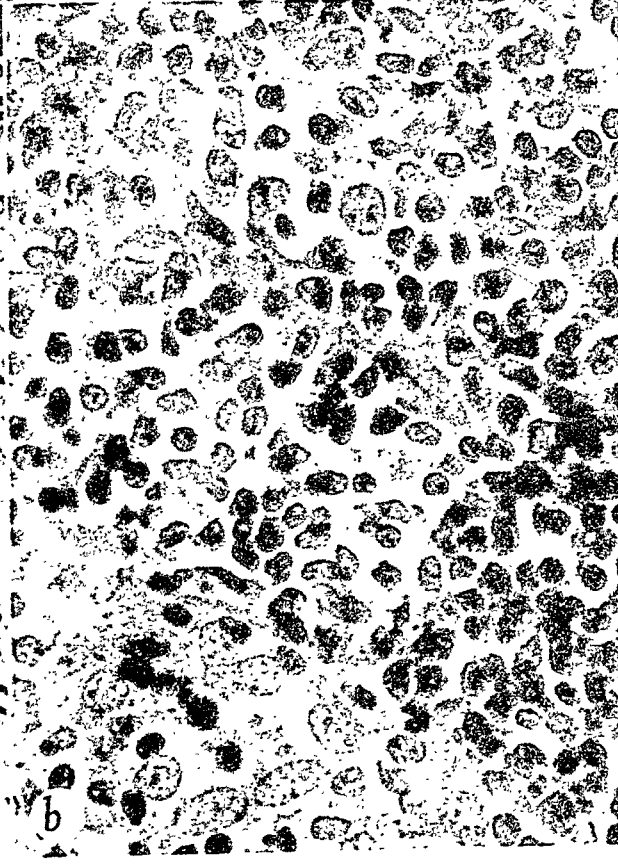
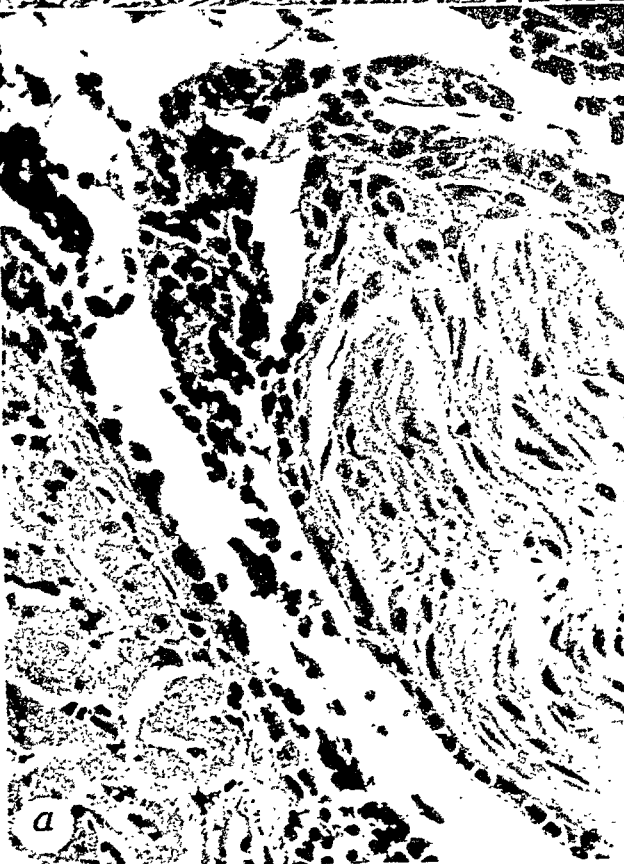
Case 14. The patient, a woman aged 32 years, was admitted to the clinic in October,

FIG. 7a. Pleomorphic cell picture of Hodgkin's disease in breast with area of fibrosis. $\times 120$. b. Note giant cell of Sternberg-Reed type, polymorphonuclear cells and mononuclear cells. $\times 865$.

FIG. 8a. Perineural lymphatic involvement in prostate by neoplastic cells. $\times 350$. b. Neoplastic cells in prostate resembling lymphosarcoma. $\times 600$.



7b



8b

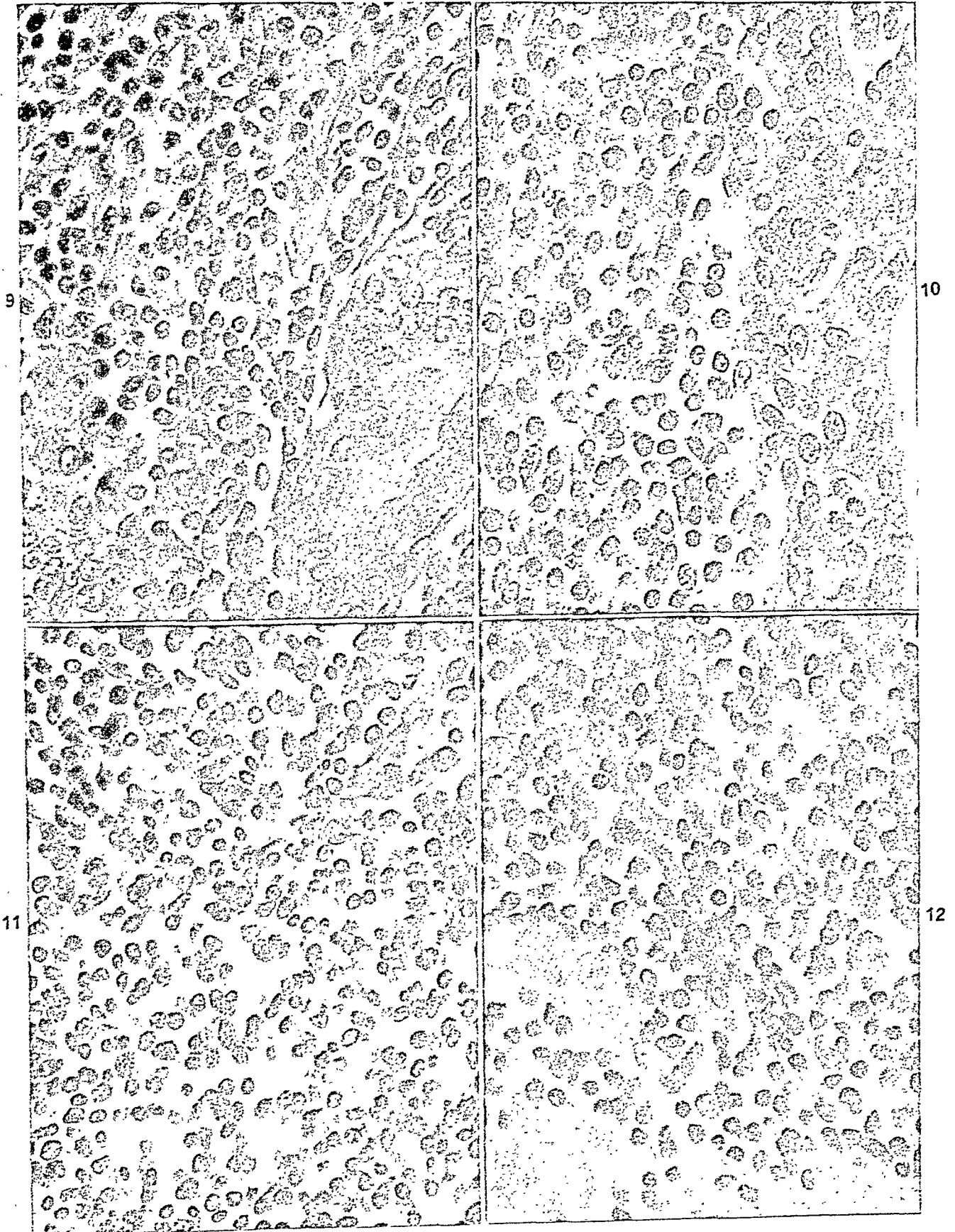
1940, complaining of weakness, loss of weight and elevation of temperature in the morning and afternoon, of two months' duration. On physical examination, a mass in the right upper quadrant of the abdomen was found. An excretory urogram was done and a diagnosis of pyelectasis and caliectasis, probably secondary to chronic pyelonephritis, was made. An exploratory operation was performed and the right kidney was removed. The kidney weighed 245 gm., and about 40 per cent of its substance was destroyed by neoplastic tissue which was composed of mononuclear cells scattered diffusely throughout the interstitial tissue of the kidney. The neoplastic cells contained fairly abundant cytoplasm and moderately chromatic nuclei, irregular in size and shape. An occasional mitotic figure was seen (Fig. 13). The cell picture was consistent with that of lymphosarcoma and since the kidney is not commonly involved with primary lymphosarcoma, it was assumed that the involvement was secondary.

Case 15. The patient, a man aged 53 years, was admitted to the clinic complaining of a feeling of discomfort in the left flank. When lying in the prone position, he was able to feel a mass in his left side. On physical examination, a large, firm, lobulated mass in the left side of the abdomen was palpated. A urogram revealed no dye in the left kidney. Left nephrectomy was done. The kidney weighed 1,920 gm. There was no involvement of the renal vein by the tumor, but there were infiltrations into the lumbar muscles and peritoneal implants of tumor tissue. About 70 per cent of the renal substance was destroyed. Histologic studies made on sections from the kidney showed masses of mononuclear cells separated by strands of reticulum fibers. The cells contained abundant pale staining cytoplasm and moderately chromatic nuclei, irregular in size and shape. There were areas of hemorrhage and blood pigment scattered throughout the section. Mitotic figures were fairly numerous and an occasional tumor giant cell was seen (Fig. 14). This case resembled a case of lymphosarcoma, but the primary site was not determined.

Case 16. The patient, a man aged 64 years, was admitted to the clinic in October, 1911, complaining of loss of weight and pain and swelling in the left groin and scrotum of five months' duration. On physical examination, shotty right inguinal nodes and a hard fixed mass in the left inguinal region extending into the scrotum were found. Left orchiectomy was done. The testis and epididymis together weighed 750 gm. The neoplastic mass appeared to be chiefly outside the testis, which seemed to be involved secondarily. Histologic studies made on sections taken from the testis revealed masses of mononuclear cells separated by reticulum fibers. These cells contained fairly abundant cytoplasm and moderately chromatic nuclei, irregular in size and shape. Mitotic figures were seen (Fig. 15). The cell picture was consistent with that of a lymphosarcoma and it was assumed that the testis was involved secondarily, although the primary site was not determined.

Case 17. The patient, a man aged 32 years, was admitted to the clinic in January, 1912, complaining of progressive enlargement of the scrotum of six months' duration. A few subcutaneous nodules over the thorax had developed one month prior to admission. One of these nodules had been removed elsewhere for diagnosis and sent here for histologic examination. It was reported as a lymphosarcoma. On physical examination, a firm, smooth, nontender right testis and numerous subcutaneous nodules scattered over the left side of the thorax and the abdomen were found. Right orchiectomy was done and a firm, white neoplastic mass, measuring 8 by 7 by 6 cm., compressing and invading testicular tissue, was found. Histologic examination made on sections taken from the testicular tumor revealed atrophy of the tubules of the testis and masses of neoplastic cells separated by reticulum fibers. The cells were medium-sized mononuclear cells with a fair amount of faintly staining cytoplasm and moderately chromatic nuclei, irregular in size and shape. Mitotic figures were fairly numerous (Fig. 16). The cell picture was consistent with that of

FIG. 9. Cells of a lymphosarcoma scattered diffusely among mammary ducts. $\times 575$.
FIG. 10. Neoplastic cells resembling lymphosarcoma beneath epithelium in bladder. $\times 575$.
FIG. 11. Neoplastic cells resembling lymphosarcoma in breast. $\times 600$.
FIG. 12. Neoplastic cells resembling lymphosarcoma in prostate. $\times 600$.



a lymphosarcoma but, again, the primary site was not determined. Additional evidence of the disease, however, was offered by the finding of lymphosarcoma in a subcutaneous nodule.

COMMENT

As seen in surgical material, lymphoblastoma is of rare occurrence in non-lymphoid organs. It is, of course, fairly commonly encountered in necropsy material but such involvement does not, as a rule, represent a diagnostic problem. We have exercised extreme caution in arriving at the conclusion that the seventeen cases in this report represent examples of this disease. It is a well known fact that a number of neoplasms under certain conditions can simulate the lymphoblastomas. Carcinomas, in particular, may assume such small cell forms as to be almost indistinguishable from some of the lymphoblastomas. It is obvious that surgical treatment should not be employed for lymphoblastomatous involvement of nonlymphoid organs; and that when the diagnosis is made, treatment preferably should be by irradiation.

SUMMARY

Seventeen operative cases are described in which there was lymphoblastomatous involvement of nonlymphoid organs. The tissues removed, either for relief of symptoms or for diagnosis, included breast, liver, prostate, testis, kidney, bladder, bone and endometrium. The pathologic process described includes lymphosarcoma, Hodgkin's disease and lymphatic leukemia.

The seventeen cases are divided into three groups. Group 1 includes those cases in which the diagnosis was established by histologic examination made on tissue removed from a lymphoid organ. This group includes seven cases:

1. Breast with lymphosarcoma, finally proved to be lymphatic leukemia by studies of the peripheral blood;
2. Liver with lymphosarcoma and node from inguinal region with lymphosarcoma;
3. Prostate with lymphosarcoma and node from cervical region with lymphosarcoma;
4. Endometrium with leukemic infiltration from a patient with lymphatic leukemia;
5. Testis with lymphosarcoma and lymph node with lymphosarcoma;
6. Testis with lymphosarcoma and necropsy findings of lymphosarcoma involving adductors of left thigh, paravertebral lymph nodes, heart, left adrenal, ribs, diaphragm and intestine; and
7. Bone with lymphosarcoma and cervical node with lymphosarcoma.

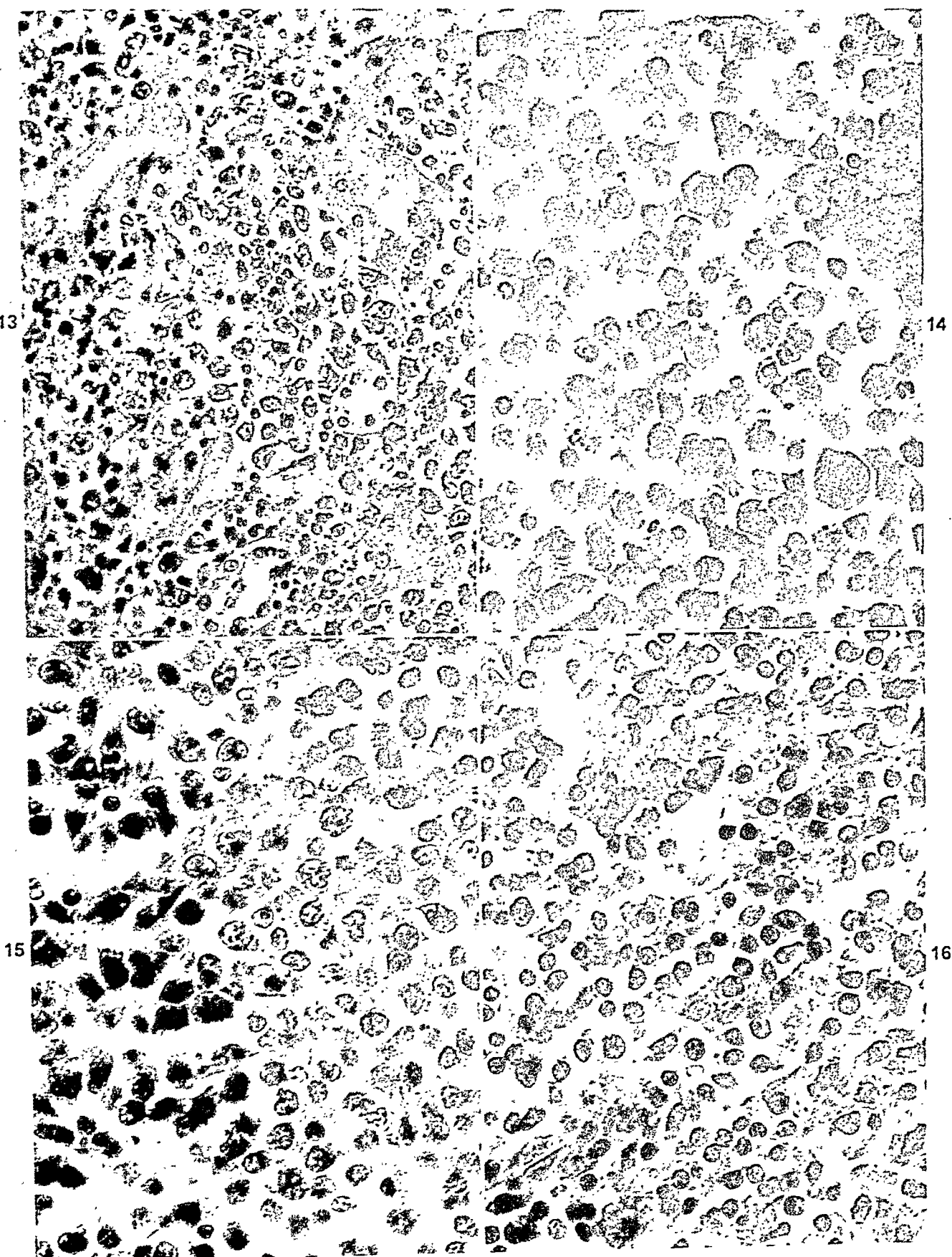
Group 2 includes those cases in which the history, physical findings, roentgenographic examination, or clinical course was strongly suggestive of a lymphoblastomatous process. There are four cases (8 to 11, inclusive):

FIG. 13. Neoplastic cells resembling lymphosarcoma among renal tubules. $\times 320$.

FIG. 14. Neoplastic cells resembling lymphosarcoma in kidney. Note giant cell. $\times 600$.

FIG. 15. Neoplastic cells resembling lymphosarcoma in testis. Note mitotic figure. $\times 600$.

FIG. 16. Histologic picture resembles that seen in Figure 15. $\times 600$.



8. Breast with Hodgkin's disease in which the history, clinical course and response to roentgen therapy were very suggestive of the disease process;

9. Breast with lymphosarcoma in which the roentgenographic examination of the thorax and physical examinations were suggestive of the disease;

10. Lymphosarcoma of prostate with generalized peripheral lymphadenopathy; and

11. Lymphosarcoma of bladder with generalized peripheral lymphadenopathy.

Group 3 includes those cases in which the only evidence of the disease was found in the surgical tissue removed but the histologic examinations of the tissue revealed malignant cells resembling those found in group 1. In this group there are six cases (12 to 17 inclusive):

12. Lymphosarcoma of breast;

13. Lymphosarcoma of prostate;

14 and 15. Lymphosarcoma of kidney; and

16 and 17. Lymphosarcoma of testis.

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A STUDY OF VARIOUS METHODS OF STOOL EXAMINATION IN THE DIAGNOSIS OF SCHISTOSOMIASIS JAPONICA*

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A study was undertaken of the results of examinations of the stools of 495 American soldiers, who had relatively light exposure to the cercariae of *Schistosoma japonicum* on Leyte in the Philippines. The purpose of the investigation was to obtain a valid basis for determining when it could be assumed that an individual with bisexual schistosomal infection was no longer passing eggs. The passage of eggs into the intestinal lumen is not consistent from day to day because (1) the female worm, not being an intraluminal helminth, relies upon parenteral oviposition; (2) eggs may be discharged irregularly from submucosal abscesses into the lumen of the intestine; and (3) it is conceivable that ineffective antimony treatment may only narcotize, rather than kill the worm, thus serving only temporarily to interrupt egg production.

MATERIALS AND METHODS

The 495 soldiers in this study were unique in that they comprised a homogeneous group of individuals from a nonendemic area exposed to the cercariae of *Schistosoma japonicum* for a short period of time, following which they were evacuated to a nonendemic area in the Zone of the Interior. The average period during which the soldiers were under observation in this installation was 121 days. Prior to this, the patients had been hospitalized overseas where they had received one, or occasionally, an additional course of fuadin and/or tartar emetic five to nine months before reaching this hospital. In 335 of these 495 men, the disease had been diagnosed overseas on the basis of positive stool examination, while in the rest, the diagnosis had been made on clinical and epidemiologic grounds alone. It is possible that some of the patients in the latter group had only a unisexual infection and would therefore pass no eggs.

At the onset of the study each patient had three stool examinations per week for a two week period. If the stools were negative, the patients were given a thirty day furlough, or if the stools were positive, the patient was placed on treatment. After the soldiers returned from furlough, their stools were again examined three times weekly for another two week period. If the stools were then negative, a twenty day furlough was given. Upon return of the soldiers from this furlough, the same routine of stool examinations was repeated for the final follow-up, thus making a possible total of 18 examinations of the stool during the period of observation.

With passage of time, it appeared advisable to alter this routine in those

* Received for publication, November 6, 1946.

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patients remaining negative, so that more consecutive specimens of stool could be examined. Accordingly, the following schedule was established: (1) 5 stool examinations each week for two weeks followed by a thirty day furlough; (2) 5 stool examinations per week for three weeks followed by a fourteen day furlough; and (3) 5 stool examinations per week for three weeks. The routine examinations made in these groups were, for the most part, by direct smear, but in addition each patient had at least 4 examinations by the sedimentation method and one by the zinc sulfate method. If a positive stool was detected during this routine, the patient was placed on treatment.

The comparative methods used are shown in detail in subsequent tables. Included are the results of extensive examinations carried out on a group of 29 individuals by the following methods: direct smear, zinc sulfate centrifugal flotation, sedimentation by gravity, modified gravity sedimentation, sedimentation by centrifugation and egg hatching. Egg counts were done on 5 specimens of formed stool found to be positive on direct smear. A total of 17,295 examinations on 12,880 stools was made by a combination of eight techniques.

The data recorded here were collected over a seven month period, six months to thirteen months after our troops first went ashore on Leyte (October 22, 1944). It may be assumed that the infections were acquired between this date and about January 15, 1945, the period during which the Army's anti-schistosomiasis program was becoming fully established. The infections in the patients under consideration could have been no less than three and no more than eleven months old when first seen here.

TECHNIQUES

In each of the first four techniques, the examination consisted of the scrutiny of all the material contained under one cover slip 22 mm. square.

Direct Smear

A fleck of feces was scraped from the surface of the specimen, emulsified in tap water on a slide and examined. In the case of formed stools, material was selected from gross blood or mucus when available. Material was taken at random from liquid specimens.

Zinc Sulfate Centrifugal Flotation Technique³

An estimated 5 gm. of feces was partly emulsified in tap water by shaking in a 60 ml. stoppered test tube, strained through one layer of wet gauze into a 20 ml. tube, washed by centrifugation at about 1000 R.P.M. for one minute until clear, resuspended in aqueous zinc sulfate solution of specific gravity 1.180, and centrifugalized at about 2000 R.P.M. for one minute. Portions of the surface film were examined with the help of D'Antoni's stain.

Sedimentation by Centrifugation⁶

After completion of washing by centrifugation as in the first part of the zinc sulfate centrifugal flotation technique, a portion of the sediment was pipetted to a slide and examined.

Sedimentation by Gravity¹

Approximately 10 gm. of feces was emulsified in tap water by trituration, strained through two layers of gauze into a 140 ml. urinalysis glass, allowed to stand for one hour, carefully decanted, resuspended in water, and then decanted at half hour intervals until the supernatant was clear. A portion of the sediment was pipetted to a slide and examined.

Modified Gravity Sedimentation Technic²

A portion of feces estimated to weigh 5 gm. was emulsified in 250 ml. 0.5 percent glycerinated water and strained through four layers of gauze into a 360 ml. wax paper cornucopia. After standing for one hour, the supernatant was decanted and the sediment resuspended in 0.5 per cent glycerinated water. Decantations and resuspensions were then repeated at half hour intervals until the supernatant remained relatively clear. Portions of the top, middle and bottom layers were each pipetted to a slide, and the three slides examined:

Egg Hatching

The sediment from the sedimentation by gravity technic was suspended in water and allowed to stand. The clear water at and near the surface was subsequently examined for miracidia at intervals up to forty-eight hours with a dissecting microscope or hand lens. A variety of modifications was used from time to time. Chlorinated tap water with pH 7.2, filtered rain water with pH 6.8, and filtered pond water, pH 7.2, were tried. The preparations were held at room temperature and at 30 C. Direct and indirect sunlight and complete darkness were tried.

Egg Counting^{7,8}

The large drop, displacement modification technic was used. A portion of feces weighing about 4 gm. was made into a homogenous suspension in 56 ml. of 0.1 N sodium hydroxide solution; 0.15 ml. of the suspension was pipetted to a slide and the eggs counted under a 22 by 40 mm. cover slip.

RESULTS

Of the 495 patients whose stools were examined in this hospital, 156 were still found to be passing eggs. The 156 men included 26 who had been negative overseas. A total of 195 patients who were positive overseas were negative under our observation.

In order to find out whether the direct smear method detected positive stools equally well during the entire period of observation, the percentage of specimens found positive during each successive month of the study is recorded in Table 1. During the first month, the percentage of positive stools in a group of 476 patients was 26.3. From the second to the fourth month, the percentage of positive stools dropped (8.9 to 9.1 per cent), while in the small groups observed in the fifth and sixth months, the respective percentages were 4.7 and 2.5. These

results do not necessarily indicate that the method becomes less satisfactory with time, but probably that with progression of time and with treatment, the percentage of individuals with positive stools diminishes. In accord with this view is the fact that, of the last 2,645 examinations by direct smear of stools of 315 patients made in a period of thirty days, only 12 specimens (from 11 patients) were found to be positive.

The relative efficiency of the several technics employed from the first through the last part of the study is indicated in Tables 2, 3 and 4. In Table 2, the results are shown of comparative examinations of the same specimens in 42 individuals with positive stools observed in the early part of this study. The direct smear method detected 88 per cent of the positive patients and the gravity sedimentation technic 38 per cent. In the final follow-up period, 80 patients

TABLE 1.

PER CENT OF SPECIMENS FOUND POSITIVE BY DIRECT SMEAR DURING EACH SUCCESSIVE MONTH OF STUDY

MONTH OF OBSERVATION	TOTAL NUMBER OF PATIENTS	PATIENTS WITH NEGATIVE STOOLS		PATIENTS WITH POSITIVE STOOLS			
		Number of patients	Number of stools	Number of patients	Number of stools	Number of positive stools	Per cent of positive stools
First.....	476	335	2,096	141	1,893	498	26.3
Second.....	417	290	1,723	127	692	63	9.1
Third.....	278	166	740	112	757	67	8.9
Fourth.....	215	114	574	101	474	43	9.1
Fifth.....	106	40	239	66	339	16	4.7
Sixth.....	24	3	11	21	119	3	2.5

were followed five times during ten days by direct smear and gravity sedimentation or sedimentation by centrifugation (Table 3). A total of 798 examinations was made with the percentage of positive stools being 0.03 and the percentage of positive patients 2.5. One of these patients was detected by sedimentation (by centrifugation) and by direct smear, the other by sedimentation (by centrifugation) alone.

Of 29 patients, each of whom previously had had 18 to 37 negative routine stool examinations, 7 were found to be positive by multiple technics. Thus, it is seen from Table 4 that by using a battery of technics, 4 additional cases were found that would have been missed if only the direct smear method of examination was used.

The negative results in these studies are of importance because they may represent failure of the technic employed, or persistent negative results may indicate that eggs are not being passed. The number of negative stools examined before the first positive stool was found in this hospital in 156 patients is shown in Table 5. This illustrates the variation in number of stools that had to be examined in a group of patients all of whom were positive, or became positive.

Data were analyzed concerning the time of appearance of negative stools in

50 patients under treatment and also concerning the subsequent time elapsed until appearance of positive stools in persons in whom treatment failed, or until a period of from 90 to 120 days after the end of treatment. There were 555 stool examinations, an average of 13 examinations per patient, in the group treated with fuadin, which included 17 persons with second courses of treatment. The range of time during which stools became negative is not known because

TABLE 2

COMPARISON OF DIRECT SMEAR AND GRAVITY SEDIMENTATION TECHNICS ON STOOL ON FOUR SUCCESSIVE DAYS IN 42 PATIENTS WITH POSITIVE STOOLS DURING INITIAL PERIOD OF STUDY

DAYS	NUMBER OF STOOLS POSITIVE BY DIRECT SMEAR	NUMBER OF STOOLS POSITIVE BY GRAVITY SEDIMENTATION
1	25	8
2	16	5
3	24	3
4	22	6
Total.....	87	22
Percentage of stools positive.....	52	13
Percentage of patients positive.....	88	38

TABLE 3

COMPARATIVE EFFICIENCY OF DIRECT SMEAR AND SEDIMENTATION TECHNICS WHEN USED LATE IN THE COURSE OF STUDY BASED ON FINDINGS IN EIGHTY PATIENTS IN FINAL PERIOD OF OBSERVATION

DAY	NUMBER OF PATIENTS WITH POSITIVE STOOLS		
	Direct smear	Gravity sedimentation	Sedimentation by centrifugalization
1	0	—	1
2	1	—	1
3	0	—	0
4	0	0	—
5*	0	0	—

* Number of examinations 798; percentage of stools positive .03; percentage of patients positive 2.5.

some remained positive throughout treatment. The average time of reappearance of positive stools in 17 patients was 51.9 days. In the group treated with tartar emetic, which included three persons who had second courses of treatment, there were 444 stool examinations during treatment, with an average of 16 such examinations per patient. The range of time during which stools became negative was from less than one to twenty-one days with an average of ten days. The average time of reappearance of ova in the stools in 3 patients was eighty-three days.

TABLE 4

INFECTIONS FOUND BY A COMBINATION OF SIX TECHNIQS IN 29 PATIENTS WHO HAD PREVIOUSLY HAD AT LEAST EIGHTEEN NEGATIVE ROUTINE STOOL EXAMINATIONS

PATIENTS*	NUMBER OF STOOLS PREVIOUSLY FOUND NEGATIVE	TOTAL PERIOD OF OBSERVATION, IN WEEKS	NUMBER OF SPECIMENS EXAMINED	TECHNIC USED					
				Direct smear	ZnSO ₄ centrifugal flotation	Sedimentation by centrifugalization	Sedimentation by gravity	Modified sedimentation by gravity	Egg hatching
1	33	16	8	0	0	1	0	0	0
2	31	15	9	1	0	0	1	0	0
3	18	8	1	1	0	0	1	1	0
4	18	11	1	0	0	1	0	0	0
5	19	15	3	0	1	0	2	1	1
6	22	22	4	2	0	1	0	0	0
7	33	20	9	0	0	1	0	0	0
Totals....			35	4	1	4	4	2	1

* Patients 1 to 5 had negative stools while overseas and patients 6 and 7, positive stools while overseas.

TABLE 5

NUMBER OF NEGATIVE STOOLS EXAMINED BEFORE FIRST POSITIVE WAS FOUND IN 156 PATIENTS

NUMBER OF STOOLS EXAMINED BEFORE FIRST POSITIVE WAS FOUND	NUMBER OF PATIENTS	ACCUMULATIVE PERCENTAGE OF POSITIVE PATIENTS
Positive on first examination	27	17
1	34	39
2 to 5	50	71
6 to 10	25	87
11 or more	20	100

TABLE 6

DATA ON 339 PATIENTS WITH SCHISTOSOMIASIS WHO HAD NEGATIVE STOOLS IN THIS HOSPITAL

Number of these patients who had positive stools overseas..... 190

TECHNIC USED	NUMBER OF EXAMINATIONS
Direct smear.....	8,134
Gravity sedimentation.....	839
Modified zinc sulfate technic.....	424
Egg hatching.....	1,015
Average number of examinations per patient.....	30.7
Average number of stool specimens examined per patient.....	24.0

Table 6 shows the results on 339 patients who were negative in this hospital but whose diagnosis had been made overseas on the basis either of coprologic or clinical evidence. On the average, 30.7 examinations on 24 specimens of stool were made on these individuals.

Egg counts on positive stools from 5 patients in the final follow-up period revealed 200 eggs per gm. of feces in one case and no eggs in the other 4. On theoretical grounds, it may be assumed that the stools of the latter 4 patients, in each instance contained less than 100 eggs per gm.⁴

DISCUSSION AND RECOMMENDATIONS

When this study was begun, there were no available data on the most effective method of stool examination in patients with relatively light infection of *Schistosoma japonicum*. This necessitated a serial study with evaluation of different technics carried out on the same specimens. As a result of this experience certain suggestions can be made concerning future examination of these patients, since the demonstration of eggs in the stool at a later date would constitute the chief criterion for administration of further antimony treatment. Liver function tests performed before, during and after treatment in these soldiers have shown that successive courses of antimony cannot be given with impunity.⁵ Furthermore, gross and histopathologic studies of two of these patients who died from external violence at about the seventh month of the disease indicated, that while there was a sprinkling of eggs in the liver, the lesions were minute abscesses that subsequently become fibrotic. These circumscribed lesions showed no evidence of fibroblastic proliferation and even if antimony treatment failed to kill the worms, it would appear impossible to have enough seeding of the liver with eggs to destroy sufficient parenchyma to produce a true cirrhosis.⁵

In view of the prognosis in these patients, it would seem advisable to give them assurance of a favorable outcome and not to hospitalize them unnecessarily. Six months after the last hospitalization in the Army, the patient should be observed again and have daily stool examinations by direct smear and sedimentation for ten consecutive days and all liver tests performed which may be indicated. If these tests and the clinical examination are negative, this routine should not be repeated until a year has elapsed and, if negative at that time, an interval of five years could probably be safely permitted to elapse before further re-examination, provided that in this interim the patient had no symptoms.

SUMMARY AND CONCLUSIONS

A total of 17,295 examinations utilizing eight technics was made on 12,880 stools from 495 American soldiers who contracted schistosomiasis japonica on Leyte in the Philippines.

Among this group there were 156 patients with positive stools, including 26 patients who had been negative overseas. A total of 195 patients who had positive stools overseas were negative under our observation.

During the first month of examination by the direct smear method, 26.3 per cent of 476 patients had positive stools, by the fourth month 9.1 per cent of 215 patients had positive stools and by the sixth month 2.5 per cent of 24 patients had positive stools.

In 42 individuals with positive stools, observation in the early part of the study

showed that the direct smear method detected 88 per cent of the positive patients and the gravity sedimentation technic 38 per cent. Twelve per cent were detected by the latter technic only.

During the final follow-up period, 80 patients had stool examinations five times during ten days by direct smear and sedimentation by gravity or sedimentation by centrifugation. The percentage of positive stools was 0.03 and the number of positive patients was two, with one being detected by direct smear and sedimentation, and the other by sedimentation alone.

Of 29 patients having from 18 to 37 previous negative stools, comparative examination by a combination of six technics indicated that if only the direct smear method had been employed, four positive cases would have been missed. The results also show that the direct smear in combination with one or more of the sedimentation methods will detect more positive stools than if any one method is used exclusively for examination.

Our results indicate that the direct smear is an efficient and valuable method of examination, but that no value can be placed on any single negative finding.

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PULMONARY EMBOLISM IN NONSURGICAL PATIENTS WITH PROSTATIC THROMBOSIS*

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Numerous articles have been published in recent years on the incidence of pulmonary embolism following surgical procedures and trauma.^{1, 4, 5, 6} The frequent occurrence of pulmonary embolism in "medical" cases has also been described,^{2, 7} although it has not been so widely emphasized. The present report furnishes data on the incidence of pulmonary embolism in nonsurgical cases and stresses the frequency of prostatic thrombosis as a source of pulmonary embolism.

This work is based upon a study of 635 consecutive autopsies which were performed during a period of five years, from January 1, 1937 to December 31, 1941, at the Pittsburgh City Home and Hospitals, Mayview, Pennsylvania.

The autopsy material at this institution is unique in several respects. Almost none of the patients had had surgical intervention just prior to death. The average age at autopsy was high (60.04 years), many patients having been institutionalized because of indigency or because of mental disease. There were 473 male patients and 162 female patients. Because of the high incidence of "medical" (nonsurgical) cases, the high average age at autopsy and the large number of indigent and mental patients, I believe that this study more closely indicates the causes of death in aged patients and more closely parallels those causes of death encountered in general practice, than do the autopsy figures from more active general hospitals having many surgical patients.

PULMONARY EMBOLISM IN ROUTINE AUTOPSY EXAMINATIONS

Pulmonary embolism occurred in 147 of the 635 patients, an incidence of 23.1 per cent. This is definitely higher than the incidence given in any report that I have reviewed. For instance, Belt² states that pulmonary embolism occurred in approximately 10 per cent of autopsies at Toronto General Hospital in Toronto, Canada, and his figure is much higher than that given in most reports. In the examination for pulmonary embolism, I followed the method, described by Belt,³ of opening the main pulmonary artery *in situ* and carefully exploring all its branches after removal of the lungs.

The division into massive and minor pulmonary emboli is necessarily arbitrary. In this study, massive embolus was diagnosed when one-half or more of the pulmonary circulation was occluded. On this basis, 43 emboli were classified as massive and 104 were classified as minor.

A definite decision as to the primary source of an embolus is frequently difficult. I have listed sources showing only definite thrombi and have not included

* Received for publication, September 9, 1946.

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probable sources, so that my percentage of emboli of undetermined origin is high. The sources for both male and female groups are shown in Table 1. In recent years, the deep veins of the legs have been stressed as the primary source of many emboli previously classified as of undetermined origin. In this study, the deep veins of the legs were explored in only a few instances, but no thrombi were found. The possibility that thrombi in the deep veins of the legs may have accounted for some of the pulmonary emboli, not only in subjects in whom no source was found, but also in some of the subjects having definite prostatic venous thrombi, must be considered. The explanation for the higher percentage of emboli of undetermined origin among females would appear to lie in failure to detect all of the thrombi in the female pelvic veins.

TABLE 1
SOURCES OF PULMONARY EMBOLISM IN 635 CONSECUTIVE AUTOPSIES

SOURCE	MALE PATIENTS (473)	FEMALE PATIENTS (162)	PERCENTAGE
Prostate.....	44		29.9
Prostate and other source.....	8		5.4
Legs.....	18	15	22.4
Heart.....	8	9	11.5
Vesical Veins.....		5	3.4
Hemorrhoidal Veins.....	2	1	2.
Spermatic Veins.....	1		0.68
Pudendal Veins.....		1	0.68
Ovarian Veins.....		1	0.68
Left arm.....		1	0.68
Sagittal sinus.....	1		0.68
Undetermined.....	15	17	21.7
Total.....	97	50	100.

A number of authors have pointed out that the percentage of pulmonary embolism becomes higher with increasing age and is usually highest between the ages of 60 and 70. My findings support this belief, but apparently the increase in percentage of pulmonary embolism does not continue after the age of 70. Of a total of 146 males and 60 females in the group aged 70 years or over, I found pulmonary embolism in 29 (19.9 per cent) males and in 16 (26.7 per cent) females. The incidence of pulmonary embolism for each sex in this age group is approximately the same as the incidence for each sex for all age groups.

The rôle of congestive heart failure in medical pulmonary embolism has been stressed repeatedly in the literature. In this series, 55 patients of the 147 showing pulmonary embolism (37.4 per cent) showed varying degrees of congestive heart failure and 37 others (25.2 per cent) showed arteriosclerotic, hypertensive, or syphilitic heart disease with coronary changes varying from mild to extreme. There was cerebral softening associated in 13 of the cases of pulmonary embolism.

PULMONARY EMBOLISM IN PATIENTS WITH PROSTATIC THROMBOSIS

Pulmonary emboli apparently arose in prostatic thrombi 44 times. Prostatic thrombosis, then, accounted for approximately 30 per cent of all emboli and represents the leading source of emboli in this report. Pulmonary embolism was found in 20.5 per cent of all males, while 45.3 per cent of all pulmonary embolism in male patients developed on the basis of prostatic thrombosis.

Prostatic thrombosis occurred in 84 male patients, which represents an incidence of 13.2 per cent in the total series and 17.7 per cent in males. In more than half of the patients with prostatic thrombi (52.3 per cent) there was associated pulmonary embolism, indicating that prostatic thrombosis is not only common, but may be dangerous.

TABLE 2
ETIOLOGIC FACTORS IN PROSTATIC THROMBOSIS IN 84 PATIENTS

Congestive failure.....	30
Acute and/or chronic prostatitis.....	9
Prostatic phleboliths.....	5
Congestive failure and prostatitis.....	4
Congestive failure, prostatitis and phleboliths.....	2
Prostatitis and phleboliths.....	1
Unexplained.....	33

TABLE 3
CLASSIFICATION OF PATIENTS WITH PULMONARY EMBOLISM ACCORDING TO SEX

SEX	SUBJECTS AT AUTOPSY		SUBJECTS WITH PULMONARY EMBOLISM		
	Number	Average age	Number	Percentage	Average age
Male.....	473	61.2	97	20.5	62.4
Females.....	162	57.2	50	30.8	60.6

Congestive heart failure was by far the outstanding etiologic factor in the production of prostatic thrombosis. Phleboliths in the prostatic plexus of veins and acute and/or chronic prostatitis were not commonly associated, while benign hypertrophy of prostate, a common finding in this series, and carcinoma of the prostate, an occasional finding, did not seem to increase the incidence of thrombosis. In a considerable number of patients (33), no causative factors for the occurrence of the thrombosis could be determined. Possible etiologic factors in the production of prostatic thrombosis in 84 patients are listed in Table 2.

In most instances, the detection of prostatic thrombosis at autopsy is quite simple, and the greatly dilated, solid veins are seen with antemortem clot protruding from the cut ends. In others, however, detection is difficult, especially when the prostatic thrombi are small. As my attention became focused on this problem by its repeated occurrence, I examined the prostatic plexus of veins with greater care. My routine examination included the making of transverse cuts across the base, posterior wall and the sides of the prostate at intervals of approxi-

mately 1 cm. I examined the severed ends of the veins and then opened the veins in longitudinal fashion. In this way I was able to detect a number of ante-mortem clots that might otherwise have escaped notice.

SUMMARY

Pulmonary embolism occurred 147 times (23.1 per cent) in a series of 635 consecutive autopsies on adults in an institution in which the patients were predominantly of nonsurgical classification and had a high average age at autopsy (60.04 years). Of the 147 emboli, 43 were classified as massive and 104 as minor.

The percentage of cases of pulmonary embolism, presumably arising on the basis of prostatic thrombosis, was surprisingly high. The association of pulmonary emboli and prostatic thrombi occurred 44 times to account for approximately 30 per cent of all cases with pulmonary emboli in the series and for 45.4 per cent of all male subjects with pulmonary emboli. Etiologic factors in prostatic thrombosis are discussed. A large number of prostatic thrombi occurred in patients showing congestive heart failure, acute and/or chronic prostatitis, or prostatic phleboliths.

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INFECTION WITH *CORYNEBACTERIUM PYOGENES* IN MAN*

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The occurrence of *Corynebacterium pyogenes* infection in man is apparently very rare. Many investigators, in reporting work on *C. pyogenes* as an animal pathogen, have pointed out the possibility of the organism being pathogenic for man. In our review of the literature, we have found only one reference of a proved case of human *Corynebacterium pyogenes* infection.^{4, 8} In this instance, Halbron and Forgeot and co-workers isolated the organism from several abscesses in a sheep herder. The patient first developed an abscess in the thoracic region. During the last six months of his life, other abscesses appeared in the left maxilla, right arm and upper regions of the right leg. The abscesses were followed by formation of fistulas and it was believed that several bones were involved. Experimental inoculation of the isolated organism was not fatal for rabbits, guinea pigs, or mice.

Since the original descriptions of *C. pyogenes* by Lucet,¹⁴ Grips,⁶ Künnemann⁹ and Glage,⁵ the organism has been isolated from calves, cattle, pigs and goats in cases of pneumonia, summer mastitis, endometritis, pyometra, abscesses, polyarthritis, nephritis and granulomatous lesions. Brown and Orcutt³ and Rolle¹⁶ made comprehensive studies of *C. pyogenes*. Merchant¹⁵ studied several species of *Corynebacterium* as pathogens in domestic animals and Lovell¹⁰⁻¹³ recently investigated the ability of the organism to produce toxin and stimulate the production of antitoxin.

The purpose of this paper is to report a case of human *Corynebacterium pyogenes* infection, occurring as a complication after frostbite of the feet. The report includes a description of the organism, with its cultural characteristics, pathogenicity and serologic reactions.

REPORT OF CASE

A 37 year old white male, a truck driver, was admitted to the hospital on January 19, 1943, complaining of "frostbite of the feet." He had been subjected to extreme cold for forty-eight hours prior to his admission.

The past history revealed the occurrence of phlebitis in 1938 and Wernicke's disease in 1939.

On admission, the patient was well developed and not acutely ill. Physical examination was largely negative. The eyes reacted slowly to light, but well to accommodation. The reflexes were normal. The feet were red, swollen, painful to pressure and no blanching was seen on deep pressure. The distal portions of all the digits were dark in color and a large number of blisters was seen over the dorsal aspects of the toes. The temperature was 102.4 F., the pulse rate 100, the rhythm regular and the blood pressure 126/60.

The erythrocyte count was 4,100,000. The leukocyte count was 13,450, with 79 per cent polymorphonuclears, 18 per cent lymphocytes, 2 per cent monocytes and 1 per cent eosino-

* Received for publication, October 8, 1946.

phils. The blood Wassermann and Kahn tests were negative. The blood sugar was 96 mg. per 100 cc.; the blood creatinine, 1.3 mg.; and the nonprotein nitrogen, 24 mg. The urinalysis was negative except for 2 plus albumin.

The patient's feet were carefully cleaned and sprayed with sulfathiazole. They were wrapped in sterile cotton and the patient was placed in a Sanders' bed. Four gm. of sulfathiazole was administered by mouth daily. Inspection of the feet three days after admission revealed considerable improvement with return of color to all portions except the toes, in which there appeared to be definite evidence of gangrene. On January 25, the patient's temperature suddenly became elevated to 103 F. from previous readings of 99 F. and 100 F. A skin rash accompanied the fever. Inspection of the feet failed to show further significant changes. X-ray films of the chest were negative. Sulfathiazole was discontinued since it was believed that the patient was sensitive to the drug. On January 28, administration of sulfathiazole was again started and the patient again reacted unfavorably. On February 1, amputation of all the toes of the left foot and four toes of the right foot was performed. The pathologic examination revealed sections of skin, supporting tissues and necrotic bone, all of which showed a rather intense inflammatory reaction characterized by large numbers of polymorphonuclear leukocytes, edema, fibrin and cell debris, with evidence of gangrene. No organisms were identified in the sections.

On February 9, the temperature curve was diurnal in type. Inspection of the feet showed the operative incisions to be satisfactorily granulating and no infection was visible. The patient continued to have daily elevations of temperature, up to 103 F. and 104 F. Culture of the blood, drawn on February 16, revealed *Corynebacterium pyogenes*. At this time, the white blood cell count was 16,000, with 73 per cent polymorphonuclear leukocytes, 19 per cent lymphocytes, 4 per cent monocytes and 4 per cent eosinophils. The blood chemical findings were normal. The urinalysis was negative. The patient was given sulfanilamide, 6 gm. daily, and several days later a blood level of 5.5 mg. per 100 cc. was recorded. No unfavorable reaction was apparent. The fever began to abate and the patient appeared improved. Inspection of the patient's feet showed evidence of infection at the operative sites and in the region of the heels. About the heels, small sinus tracts had developed with seropurulent drainage. Cultures taken from the feet revealed the identical organism as that isolated from the blood. On March 10, the patient's hemoglobin had dropped to 55 per cent and the red blood cell count to 3,000,000. He received several blood transfusions. The patient's local condition did not improve. The temperature curve returned to normal and the sulfanilamide was gradually reduced to small doses. X-ray films of the feet showed minimal destruction of the os calcis of the left foot. There was no evidence of peripheral arterial disease. Clinically, a low grade infection was still present in the incisional areas and heels, and the sinus tracts in the heels continued to drain. Repeated blood cultures were all negative. The patient was discharged from the hospital on May 1, and was able to walk with the aid of crutches. He returned to the hospital for plastic repair of the extremities. On October 20, 1944, cultures from the areas of the feet previously treated still revealed *C. pyogenes* present.

BACTERIOLOGIC FINDINGS

The organism was first isolated from a routine blood culture taken on February 16, 1943. Fifteen cc. of blood was drawn and inoculated into mediums by distributing 5 cc. into brain broth, 5 cc. into brain heart infusion broth, 1 cc. into thioglycolate broth and 2 cc. each into plain agar and dextrose agar pour plates. In three days, the flask of brain heart infusion showed evidence of growth in the form of two or three individual colonies among the blood cells in the bottom of the flask. Microscopic examination showed pleomorphic, small gram-positive bacilli. The bacilli varied in length from 0.3 to 2 microns. For the most part, they took a uniform stain, but a few showed barred forms. They were arranged

in clumps or in palisades, like diphtheroids. On the fourth day, one deep colony with a zone of hemolysis, approximately 2 mm. wide, appeared in the dextrose agar pour plate. With Gram's stain, the organisms of the colony showed the same microscopic characteristics. Subsequent blood cultures taken February 24, March 2 and April 5, 1943, were negative.

On February 25, pus from a draining sinus on the patient's right foot was cultured. It showed a heavy growth of the same gram-positive bacillus and a light growth of hemolytic *Staphylococcus aureus*. On April 27, cultures were taken of pus from a draining sinus in each foot. Both cultures showed findings identical to the previous culture from the right foot.

Repeated cultures from the sinuses of the feet were taken during the next eighteen months, when the patient visited the out-patient clinic. Cultures of the patient's feet taken on August 2, September 9 and October 20, 1944, still showed the presence of the predominant organism as *Corynebacterium pyogenes*, in association with hemolytic *Staphylococcus aureus*.

Morphology. Further subcultures on mediums containing blood or blood serum showed the same pleomorphism as originally seen. The organism appeared as short, thin gram-positive bacilli, or cocco-bacilli, often in palisade arrangement similar to that of diphtheroids. On staining a twenty-four hour culture, most of the organisms showed gram-positive characteristics, but occasionally some appeared gram-amphophilic. Individual bacilli frequently exhibited irregularities of density in staining, producing a similarity to the barred forms of *C. diphtheriae*. They varied in size from 0.3 micron in the coccoid form, to 2 microns in the longer form. They showed little evidence of clubbing and were nonmotile.

Cultural characteristics. The organism seemed to grow equally well aerobically and anaerobically. One of the predominant characteristics was its ability to hemolyze blood, producing a zone of hemolysis when grown on human or rabbit blood agar. The colonies, when grown on blood agar, appeared in twenty-four hours as small pin point colonies, with zones of hemolysis, approximately 2 mm. wide. They were similar to the colonies of beta-hemolytic *Streptococcus*. In forty-eight hours, the colonies appeared slightly larger and opaque, with a dry granular surface and larger zone of hemolysis. As the colonies aged, they became medium-sized with increased opacity and granularity. The zones of hemolysis increased to 5 or 8 mm. in width.

The organism reproduced well only on, or in, mediums containing blood or blood serum. There was no growth on plain agar. There was no pigment production. It grew in plain broth only in association with the hemolytic *Staphylococcus aureus*. The morphology and growth was excellent on Loeffler's medium. It grew in thioglycollate broth with added blood serum. For growth in any of the fermentation broths, it was necessary to add blood serum.

Fermentation tubes were employed using 0.1 per cent bromocresyl blue and 1 per cent sugar and an enrichment of blood serum. The organism showed fermentation with acid production from dextrose, sucrose, lactose, arabinose, trehalose, xylose, maltose and levulose, while mannite and inositol were not

fermented. The following chart gives the sugar fermentations of our strain and of various other strains of *C. pyogenes*, as reported by Bergey,² Merchant¹⁵ and Lovell.¹³

Subcultures of the organism isolated from the blood and sinuses of the feet of the patient were sent in March, 1943, to the National Institute at Bethesda, Maryland, for identification. On April 19, 1943, the Institute reported the identity of the organism in these cultures as *Corynebacterium pyogenes*.

SEROLOGIC REACTIONS

The patient's serum taken on August 2 and September 9, 1944, was tested for antitoxin content according to Lovell's¹² method with known *Corynebacterium*

TABLE 1

REACTION OF DIFFERENT STRAINS OF *C. pyogenes* WITH RESPECT TO FERMENTATION IN SUGAR MEDIUMS, AS REPORTED BY VARIOUS AUTHORS

MEDIUM	OUR STRAIN	BERGEY ²	MERCHANT'S STRAIN	LOVELL'S STRAIN
Dextrose.....	+	+	±	+
Sucrose.....	+	+	+	— (few strains +)
Lactose.....	+	+	+	+
Arabinose.....	+	+	—	+
Mannite.....	—	—	+	— (few strains +)
Trehalose.....	+	—	—	+
Inosite.....	—	—	—	+
Xylose.....	+	+	—	+
Litmus milk.....	acid, slight coagulation	acid, slight coagulation	(few strains +) coagulation and diges- tion	coagulation and digestion
Maltose.....	+	+	+	+
Levulose.....	+	+	+	+

pyogenes toxin. These serums failed to show any antitoxin present other than in the undiluted state, which is within the range of normal as determined by Lovell in his work on human serums. Unfortunately, serums previously obtained from the patient were discarded before antitoxin studies could be carried out.

A rabbit was first inoculated intravenously on May 10, 1943, with 1 cc. of a forty-eight hour culture of the organism isolated from the patient. The animal died during the third night. Cultures taken from the rabbit were negative and microscopic examination of the rabbit's tissues showed no specific lesions relating to the inoculated organisms.

Several animals were inoculated October 27, 1944, with a forty-eight hour culture of the organism isolated from pus from the patient's foot on October 20. The animals were first bled and then inoculated as follows: one rabbit, 1 cc. intraperitoneally; one rabbit, 1 cc. intravenously; one guinea pig, 0.5 cc. intra-

peritoneally; one guinea pig, 0.5 cc. subcutaneously; 2 mice, each 0.2 cc. intra-peritoneally; one mouse, 0.2 cc. subcutaneously. The animals developed no particular symptoms, nor did any of the animals die. Three weeks after inoculation, the animals were again bled and killed, but no abnormalities were observed on autopsy. Tissue sections taken for microscopic study failed to reveal specific lesions.

Antitoxin determinations were run on the serums obtained before, and three weeks after, the inoculation of the animals with the isolated organism. The antitoxin determinations were made according to Lovell's¹² method, with some modification.

Dr. Lovell supplied the known *Corynebacterium pyogenes* toxin and antitoxin, which had been found to have the following values: dried *C. pyogenes* toxin,

TABLE 2
PROTOCOL OF PRIMARY TITRATION OF *C. pyogenes* TOXIN AND ANTITOXIN

TUBE	AMOUNT OF <i>C. pyogenes</i> ANTI-TOXIN ADDED: 1:50 DILUTION OR $\frac{1}{4}$ U. PER 0.5 cc.	AMOUNT OF <i>C. pyogenes</i> DRIED TOXIN ADDED: 0.06 gm. PER 10 cc. SALINE		1 PER CENT RABBIT CELLS	
1	0.5 cc.	0.5 cc.	Incubate 1 hour at room temperature	1 cc.	Incubate 1 hour at 37 C.
2	0.5 cc.	0.5 cc.		1 cc.	
3	0.5 cc.	0.5 cc. dilution 1:2		1 cc.	
4	0.5 cc.	0.5 cc. dilution 1:4		1 cc.	
	continue serial dilutions of toxin, as above				
Control I.....	0.5 cc. plus	0.5 cc. saline		1 cc.	
Control II.....		1.0 cc. saline		1 cc.	

approximately 0.006 gm. = 1 Lh dose; *C. pyogenes* antitoxin, 1 cc. = 25 "x" units. A primary titration was done, using a fixed amount of antitoxin in each tube and varying the amount of toxin to determine what dilution of toxin would give a 2 plus hemolysis of 1 cc. of 1 per cent rabbit cells with $\frac{1}{4}$ "x" unit in 0.5 cc. of antitoxin.

That dilution of toxin which showed a 2 plus hemolysis with $\frac{1}{4}$ "x" unit of antitoxin was then used to titrate the various animal serums. The animal serums were heated forty-five minutes at 56 C. Then each serum was titrated making serial dilutions from 1:2 to 1:2048, and using 0.5 cc. quantities; to each dilution was added 0.5 cc. of determined toxin dilution. Tubes were incubated one hour at room temperature; 1 cc. 1 per cent rabbit cells was added to each tube, and then the tubes were incubated one hour at 37 C. The 2 plus hemolysis end point was read. The following controls were used: control I, saline and rabbit cells; control II, $\frac{1}{4}$ "x" units antitoxin, saline and rabbit cells; control III, duplicate 2 plus hemolysis tube of $\frac{1}{4}$ "x" units antitoxin, dilution of toxin

determined in primary titration and rabbit cells. Two normal rabbit serums in serial dilutions were also run as controls.

None of the serums of animals taken before inoculation showed any titer. Only the serum of the rabbit which had been inoculated intraperitoneally with 1 cc. of a forty-eight hour culture of the organism showed a titer. This serum showed a content of *C. pyogenes* antitoxin of 512 "x" units per cc. This demonstrated that our organism was capable of inducing formation of specific antibody, which in turn could be titrated with a known *C. pyogenes* toxin. Thus the organism was identified serologically as *Corynebacterium pyogenes*.

C. pyogenes isolated from animal infections, has been found to be pathogenic for rabbits, mice, rats and guinea pigs.^{3, 13, 15} But the strain isolated by Forgeot *et. al.*⁴ was not fatal for laboratory animals. It is possible that strains vary a great deal in virulence. It may be postulated that this strain of the organism which we isolated lost some virulence because of the chronicity of the infection, the chemotherapy employed before cultures were taken, or because of the serologic studies made.

COMMENT

The present report offers evidence that *Corynebacterium pyogenes* is pathogenic for man. The organism invaded tissue already weakened and damaged by frostbite and produced an infection having both acute and chronic phases. It produced further local tissue damage and bone necrosis. It was hazardous as a blood stream invader, since the septicemia which occurred in this case, offered opportunities for metastatic lesions to occur. Presumably, however, the sulfonamides acted to prevent the establishment of other foci. That the organism can involve bone was shown by the x-ray findings.

The necrotic lesions, the sinus formation and the involvement of the bone in this case appeared to be similar to the types of lesions which were produced in the patient described by Forgeot *et. al.*⁴ Both of these human strains are similar in the types of lesions produced and in their inability to cause a fatal infection in laboratory animals.

This strain of the organism was able to grow and produce localized areas of degeneration. The failure to demonstrate lesions in inoculated animals is possibly due to the low virulence of our strain, or to virulence that was altered by previous chemotherapy. A notable feature of the infection in this human case was its long chronicity, extending over many months.

SUMMARY

An instance is reported of a human infection with *Corynebacterium pyogenes* which constitutes the second such infection reported in the literature. In the present infection the tissue showed previous circulatory damage. There was also a septicemia. Serologic examination showed production of toxin by the organism and the ability of the latter to stimulate formation of antibody.

Acknowledgments. The authors wish to express their thanks to Dr. I. A. Merchant, Iowa State College, Ames, Iowa, for supplying us with cultures of *C. pyogenes* used for

comparison, and to Dr. Reginald Lovell, Royal Veterinary College, London, for supplying us with the *C. pyogenes* toxin and antitoxin used in our determinations.

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DIFFUSE OSTEOID REPLACEMENT OF THE BONE MARROW

REPORT OF CASE*

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Intravital bone marrow studies have become an important aid in clinical diagnosis, particularly in the search of etiologic factors underlying unexplained peripheral hematopathologic disorders. Since some diseases of this blood forming organ have not been discovered by physical findings, roentgenograms, or elaborate laboratory data, we have been led to the adoption of "sternal aspiration" as a routine procedure^{8, 9, 12-15} in our institution. While sufficient marrow tissue is generally obtained with the aspiration method, cases are encountered that require the use of the sternal needle as an instrument to procure an adequate specimen for biopsy. Among the morbid conditions of the marrow subject to biopsy are Hodgkin's disease (granulomatous type), metastatic carcinoma, Ewing's tumor and malignant hemangio-endothelioma.

It is the purpose of this paper (a) to report a case in which the organopathology, namely "diffuse osteoid replacement of the bone marrow," was demonstrated by the method here described and (b) to re-emphasize the value of the needle method to furnish material for biopsy when one fails to obtain marrow tissue (sternal marrow units) with the aspiration procedure. In the writer's file of more than several thousand bone marrow specimens, the combined files of the Department of Pathology of the Minneapolis General Hospital and University of Minnesota, as well as in the available literature, no identical or similar case is recorded.

REPORT OF CASE

A critically ill 73 year old woman of Norwegian stock was admitted on March 20, 1945, to the medical service of the Minneapolis General Hospital. She had lobar pneumonia and a severe anemic state. She expired March 22, and an autopsy was performed.

The essential laboratory data were as follows: hemoglobin, 8.0 gm. per 100 cc.; erythrocytes, 2,700,000 per cu. mm.; leukocytes, 13,600 per cu. mm.; differential count (percentage of cells), neutrophils 67, lymphocytes 12, monocytes 6, eosinophils 3, basophils 1, metamyelocytes 8, neutrophilic myelocytes 3, reticulocytes 1. One normoblast was observed per 100 leukocytes. The mean corpuscular diameter of the erythrocytes was 7.6 microns. The blood Wassermann test was negative.

The past history revealed that the patient had been hospitalized in 1938 with a Colles's fracture of the right wrist that resulted from a fall on an icy street three days before she presented herself for treatment. The fracture caused only moderate discomfort and was reduced without surgical intervention. Recovery was uneventful. No anemic state was present at that time. She was the eldest of three children and as far as she knew, her brother and sister were in good health, although she had had no contact with either one for many years. Her parents died of old age and neither came to autopsy.

* Received for publication, November 2, 1946.

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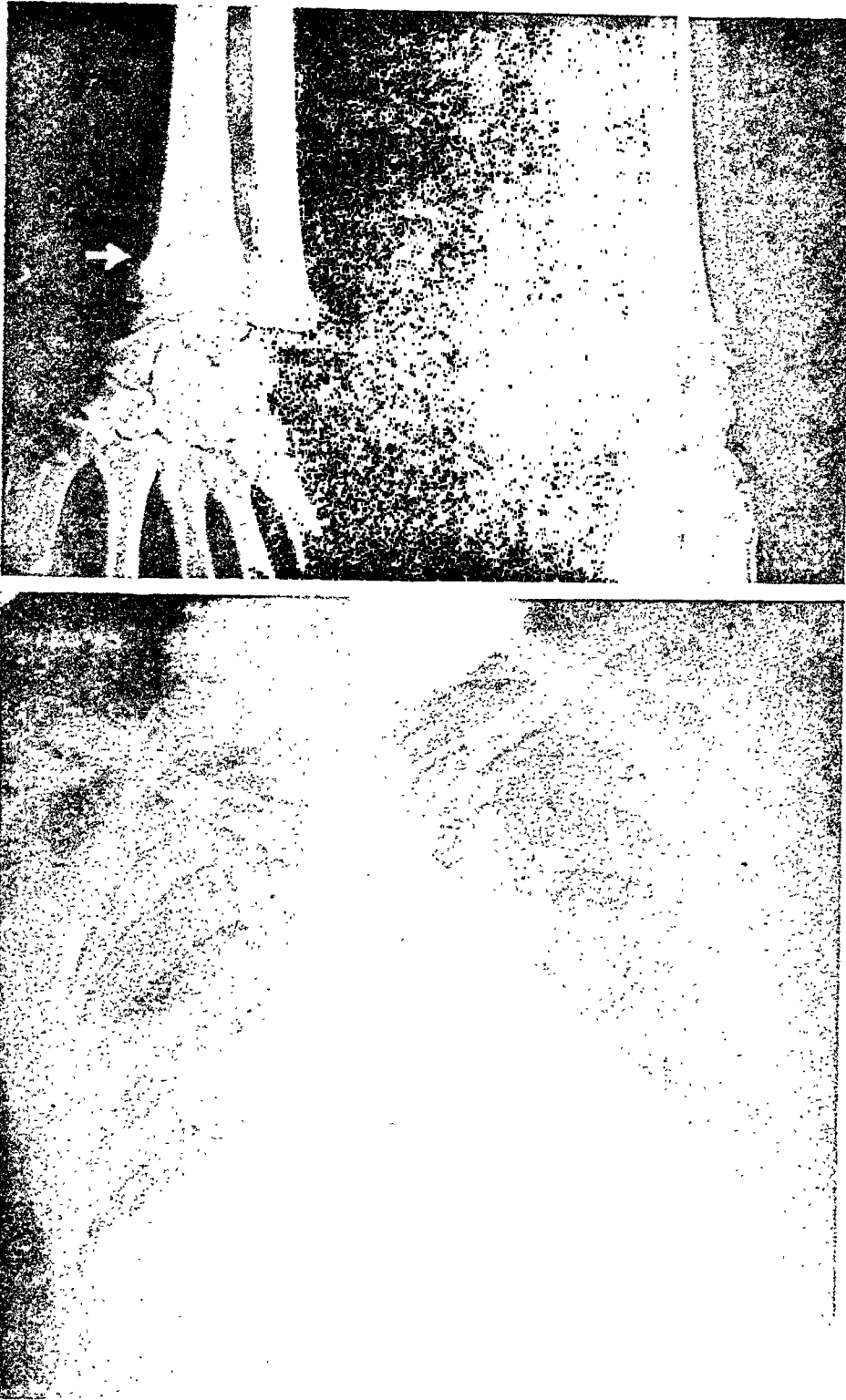


FIG. 1. Upper roentgenogram made in 1938 shows the Colles's fracture of the right radius before it was reduced (white arrow). Lower roentgenograms made in 1945 show the left and right sides of the chest. Note the marked degree of osteoporosis, which is in sharp contrast to the opacity of the bones in Albers-Schönberg disease.

Because of the presence of an unexplained severe anemic state at the time of her recent admission, a sternal aspiration was performed. A standard sternal aspiration needle

(Fig. 2) was employed.^{12, 14} The anterior lamina of the sternum was easily penetrated. But in spite of proper technic, no marrow tissue could be aspirated. A small core of marrow tissue was obtained by the method described here.

NEEDLE METHOD FOR BIOPSY

The needle is inserted into the anterior lamina and medullary cavity of the sternum, and while still *in situ* and without stylet, is turned several more millimeters into the medullary cavity. An airtight glass syringe reinforced with a metal adapter is attached to the needle head and suction applied while the needle part is turned into the medullary cavity. The negative pressure is maintained while the instrument is withdrawn in order to hold aspirated tissue in the lumen of the needle. A tissue core thus obtained may be expelled from the lumen by introducing the stylet and gently pushing out the specimen into a fixing fluid (consisting of 1 part of neutral formaldehyde, 40 per cent saturation and 9 parts of physiologic sodium chloride), or forcing fixing fluid directly into the lumen of the needle. When imprints or smears of the tissue are desired, it is best to place the unfixed specimen upon a paraffin coated glass plate in order to permit easy transfer of a small part to a wood applicator and glass slide, respectively.¹⁶

HISTOPATHOLOGY OF THE BONE MARROW

Histologic preparations of the tissue core revealed that the specimen consisted of several fragments of osteoid, fibrous tissue, some erythroid and myeloid cells and fat. It was deemed advisable to determine whether the observed pathologic process was restricted to the aspirated area, or was diffuse in character, in order to classify the morbid condition correctly. Thus, aspiration of marrow from several bones was planned, but the sudden death of the patient nullified this intention. At autopsy, several specimens were taken from the sternum, ribs (one of which showed a well healed fracture), vertebrae, left and right humerus, right radius near the healed Colles's fracture and the middle portion of both femurs. All bones, with the exception of the latter, could be cut easily with a knife. Serial sections of the bone specimens showed the following histopathology. The cortex varied in thickness, the bone trabeculae in some areas were thicker than normal and closely spaced, whereas in other areas they were normal in size and spacing. Osteoid tissue was scattered throughout the medullary cavity, the amount varying considerably in the individual sections taken from a single bone. The specimens from femur showed various-sized osteoid islets, a conspicuous quantity of fat, some hematopoietic islets and a small amount of fibrous tissue.

The sections of all other bones showed areas of marrow hyperplasia alternating with areas of various degrees of hypoplasia and fatty metamorphosis. Fibers and fibrous cords, which appeared to be thickened and fused reticulum fibers, surrounded bone trabeculae and also coursed between osteoid islets. Within broad fibrous bands and knobs, small amounts of calcium were deposited. Figure 3 shows clearly the over-all histopathology just described. Sections of liver, spleen, lymph nodes, kidneys and other routine autopsy material revealed nothing of note. It is of particular interest that the organopathology was not



FIG. 2. Aspiration needle used (K-S sternal needle made by V. Mueller and Co., 408 South Honore Street, Chicago 12, Illinois).



FIG. 3. Average serial section of the sternum about 7 microns thick. The bony trabeculae are somewhat thickened and closely spaced. The diffuse replacement of bone marrow by osteoid tissue, small amounts of fibrous tissue, and moderate fatty metamorphosis of the scattered hematopoietic islets is well shown. The histopathology in the other flat and long bones did not deviate appreciably from this over-all pattern. Hematoxylin and eosin stain. $\times 400$.

revealed by the appearance of various bones in the roentgenograms which were interpreted as characteristic of "senile osteoporosis" (Fig. 1).

DISCUSSION

The case reported here suggests a genetic relationship to Albers-Schönberg disease. To classify the observed condition as a biologic variation of either the dominant or the recessive form of this hereditary and clinical entity, however,^{1-6, 10, 11, 17, 18} is subject to approval by students of this disease, but one may hypothesize that the "diffuse osteoid replacement of the bone marrow" is a mutation of the aforementioned disease. Analysis of the over-all histopathology of Albers-Schönberg disease revealed that the morbid process resolves itself into the following features: (a) endochondral ossification is disturbed as soon as bone formation begins; (b) there is continuous production of true bone, thickening of bone trabeculae and production of fibrous tissue; (c) there is loss of elasticity of the bones, causing brittleness; and (d) finally, the progressive thickening of the cortex and encroachment of the medullary cavity by newly formed bone makes for the extreme hardness and characteristic opacity (structureless appearance) of the bones in the roentgenogram. The described pathology of the marrow and the state of the bones in the present instance deviate from the over-all pattern of Albers-Schönberg disease because: (a) osteoid tissue and not true bone replaced the marrow substance; (b) the appearance of the bones in the roentgenogram was that of "senile osteoporosis;" and (c) the bones could be cut easily with a knife. In view of the fact that osteoid, fibrous and lipid tissue replaced the marrow, and that this process apparently caused no specific clinical symptoms, the case presented here may be considered as a heretofore unrecognized mild form of Albers-Schönberg disease, keeping in mind that the hereditary character of the organopathology is not established. It is believed that the described morbid process is not a variable of the condition known as "myelosclerosis" or "myelofibrosis," which is characterized by some true bone formation and replacement of the marrow by dense fibrous tissue, splenomegaly, extramedullary hematopoiesis and a more or less conspicuous leukemoid reaction.^{7, 11}

SUMMARY

A case with "diffuse osteoid replacement of the bone marrow" in a 73 year old woman is reported. The organopathology was demonstrated during life by a method which is described for furnishing material for biopsy. The diffuse character of the morbid process was elucidated by the microscopic examination of bones obtained at autopsy. The bones could be cut easily with a knife.

The appearance of the bones in the roentgenogram was that of "senile osteoporosis." The possibility of a genetic relationship of the organopathology to Albers-Schönberg disease is discussed.

Diffuse osteoid replacement of the bone marrow is apparently rare. No diagnostic clinical symptoms revealed the condition.

The value of intravital bone marrow studies and the importance of keeping in mind primary diseases of the bone marrow as an etiologic factor of obscure and refractory anemic states is clearly shown.

Acknowledgments. I wish to thank Dr. G. E. Fahr, Head of the Department of Internal Medicine, Minneapolis General Hospital, for his permission to publish the case, and Dr.

E. T. Bell, Head of the Department of Pathology, University of Minnesota, for his cooperation. The photomicrograph was made by Mr. H. W. Morris, medical photographer, University of Minnesota.

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ERYTHROID MULTIPLE MYELOMA*

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REPORT OF CASE

Clinical Data

R. T., a 60 year old white male, a riverboat engineer, was admitted to the U. S. Marine Hospital, Pittsburgh, Pennsylvania, May 19, 1945. His family history was noncontributory. His past history revealed that he had been free of illness, except for typhoid fever 50 years ago, and that he had been a heavy drinker. His illness had started six months before admission with a head cold and generalized aching. The aching persisted and made it necessary for him to give up his work. The pains were sharp, severe and steady. He said they were like "red-hot knives" lying between his scapulae, along his spine and in his arms and legs. He also experienced some pain in his chest which was aggravated by coughing. He complained of extreme and progressive weakness so that he had been unable to take care of himself at home. He had lost about 50 pounds in weight. His only other complaint was nocturia and frequency every two hours.

Physical examination revealed an obviously anemic, frail, tired-looking, poorly nourished man who appeared at least 10 years older than his stated age. The following positive findings were present. Oral hygiene was poor. The heart was slightly enlarged to percussion and a short, harsh apical systolic murmur was audible. The blood pressure was 120/65. There were fine crepitant râles in the lower lobe of the left lung. The abdomen was normal except for gaseous distension. All reflexes were sluggish. Vibratory sense and sense of position were present. The outstanding physical finding was an unusual degree of tenderness of all the bones, especially of the ribs and sternum. The slightest pressure caused exquisite and very real pain. No irregularities of the bones and no tumors were present.

The urine contained 4 plus albumin, 12 to 20 white blood cells per high power field and many granular casts. No Bence-Jones protein was found at any time during his hospitalization. The blood showed profound anemia with 1,520,000 erythrocytes per cu. mm.; hemoglobin, 23 per cent (Sahli); color index, 0.7; and volume index 1.2. The leukocyte count was 16,800 per cu. mm.; neutrophils, 52 per cent with a marked shift to the left and lymphocytes, 48 per cent. Four normoblasts per 100 leukocytes were seen and slight poikilocytosis and anisocytosis were noted. The nonprotein nitrogen was 48 and the serology was negative. X-ray films of the bones of the chest, spine and pelvis showed moderate diffuse demineralization without specific lesions. Gastrointestinal x-ray series showed atrophic gastritis, but gastric analysis showed normal acidity. He received heavy doses of sedatives and many types of narcotics in a vain attempt to control

* Received for publication, November 9, 1946.

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the pain. In addition to many other supportive measures, he was given large doses of liver extract, up to 50 units a day intramuscularly, and iron orally. A transitory rise was obtained in the reticulocyte count, with a peak of 6.3 per cent on June 2, after which it leveled off at 3 per cent. The erythrocyte count ranged between $1\frac{1}{2}$ and 2 million and the differential count showed only slight change, the shift to the left of the neutrophils persisting without showing a definite leukemic picture. There was, however, an increase in the number of normoblasts which ranged between 15 and 19 per 100 leukocytes and the erythrocytes showed more hypochromia, anisocytosis and poikilocytosis.

The very severe bone tenderness had made it impossible to do a satisfactory sternal puncture. Finally, on June 24, with liberal novocaine infiltration, some bloody fluid was obtained from the sternal marrow cavity. Unusual cells were seen, which were classified as atypically staining, small lymphocytes and the marrow was considered to be within normal limits except for the preponderance of lymphocytes. After autopsy, the slides were reviewed and the following differential count was made: myeloblasts, 2; promyelocytes, 2; myelocytes, 6; neutrophils, 31; eosinophils, 2; basophils, 0; lymphocytes, 9; plasma cells, 0; monocytes, 2; reticulo-endothelial cells, 0; megakaryocytes, 0; megaloblasts, 2; myeloma cells, 23; unclassified cells, 21; normoblasts, 7; normocytes, 5.

The cells classified above as myeloma cells were small and had very dark, almost uniformly black nuclei resembling those of normoblasts. Some had a small amount of colorless, hyaline cytoplasm and others had no discernible cytoplasm.

Repeated attempts to find satisfactory donors for transfusion failed. The patient's blood was typed as group A, but it was found that his serum agglutinated the cells of each of approximately two dozen donors that were tested. On August 4, his leukocyte count abruptly rose to 73,900 and showed the following differential count: neutrophils, 34; stab cells, 30; lymphocytes, 9; monocytes, 4; myelocytes, 8; promyelocytes, 13; myeloblasts, 2. On August 17, he started to vomit and shifting dulness was elicited in the abdomen. His pain was still primarily localized to his bones. Steady deterioration in his condition continued. On August 24, he fell in attempting to get out of bed and sustained a fracture of the neck of the left humerus. He died the following day.

Autopsy Findings

A complete autopsy was done thirty-six hours after death. The body showed advanced emaciation, cachexia and slight cyanosis. The anatomic diagnoses were: myeloma, apparently of erythroblastic type, diffusely involving bone marrow and spleen; myeloma nodule attached to third dorsal vertebra; pathologic fracture of surgical neck of left humerus; acute gangrenous appendicitis with perforation and generalized peritonitis; pulmonary congestion and edema; old cerebral thrombosis, internal capsule; myocardial fibrosis and atrophy; nephrosclerosis; and adenomatous hyperplasia of prostate.

The positive gross and microscopic findings were:

Brain. A pink area, 1 cm. in diameter, was present in the internal capsule

and, on section, showed a large number of thin-walled blood vessels filled with hyaline material.

Heart. Weight 510 gm. Myocardium was dull, reddish brown and flabby; microscopically, it contained focal areas of scarring.

Pleura. In the left pleural cavity projecting from the left lateral aspect of the third dorsal vertebra, a smooth, firm, gray neoplastic mass measuring 2 x 1 cm. was found attached to the bone without apparently invading its substance. Histologically, it was composed of cells identical with those which will be described in the bone marrow. Binucleate and multinucleate forms were seen occasionally and a few plasma cells were noted. These cells were supported by a loose connective tissue framework, and in one corner of the nodule a discrete ganglion was imbedded.

Lungs. The upper lobes were normal, while the lower lobes showed marked congestion. Microscopically, the lower lobes showed, in addition to the extreme congestion, alveoli filled with serum, pigment-containing leukocytes and a few lymphocytes and neutrophils.

Liver. Weight 967 gm. The capsule was thickened and, histologically, the cells immediately beneath the capsule had undergone fragmentation and nucleolysis. The parenchyma was otherwise normal except for slight fibrosis.

Spleen. Weight 247 gm. Moderate autolysis was noted. The follicles were small and the red pulp was depleted of erythrocytes. Large numbers of cells similar to those in the bone marrow were scattered throughout.

Kidneys. The combined weight was 390 gm. Grossly, the cortical surfaces were finally granular. Microscopically, there was generalized fibrosis, several glomeruli showed hyalinization, and the arteries and arterioles showed moderate sclerosis.

Prostate. A small nodule in the upper portion of the right lobe showed hyperplastic changes but no evidence of neoplasia.

Gastrointestinal tract. The peritoneal cavity contained 250 cc. of thick, grayish white, malodorous pus. The stomach and intestines were greatly distended by gas and liquid material. The appendix was long, retrocecal and showed gangrenous necrosis in its terminal 2 cm. A definite area of perforation, exuding thick yellow pus, was found near the tip. Microscopically, there was extensive necrosis and acute inflammatory reaction of the peritoneum and of the appendix, especially in the immediate neighborhood of the perforation.

Skeletal system. All the bones were very fragile, the ribs being easily cracked with the fingers. There was obvious thinning of the cortices and correspondingly marked hyperplasia of the bone marrow. A transverse fracture of the surgical neck of the left humerus surrounded by a large hematoma was found and in this area the bone marrow was particularly hyperplastic. Microscopically, the bone marrow showed moderate autolysis. Most of the marrow was composed of an acidophilic hyaline substance in which were imbedded large numbers of cells of several types. No specific arrangement or pattern of these cell types was noted. The predominating variety was a small cell with a compact, round, intensely basophilic or almost black nucleus with little or no cytoplasm. The small

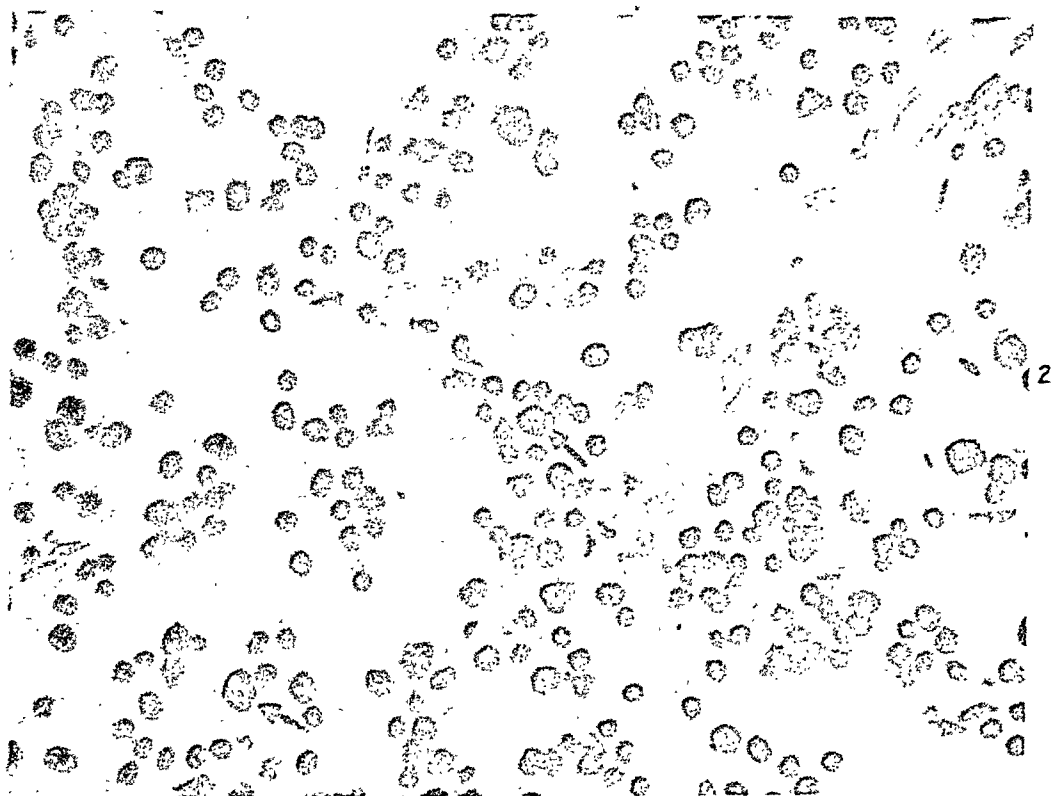
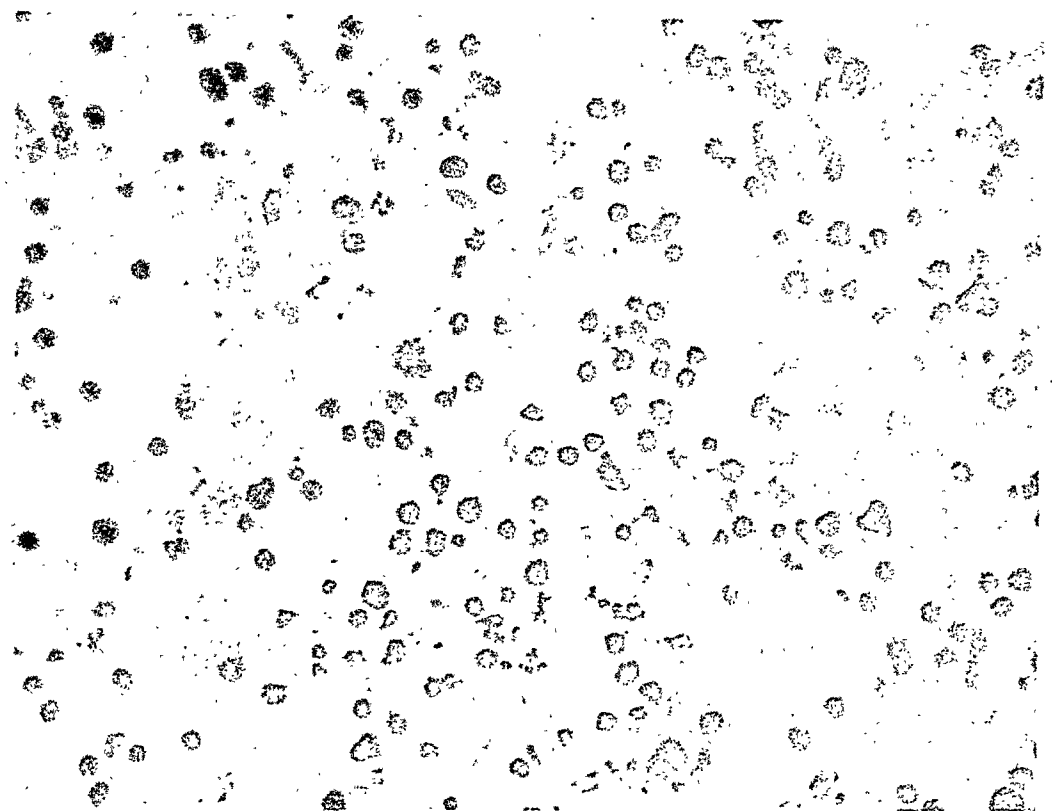


FIG. 1. Bone marrow showing sheet of homogenous eosinophilic matrix with nuclei of erythroblastic series. Occasional plasma cells and mitotic figures may be noted. $\times 550$.

FIG. 2. From pleural nodule, showing erythroid cells including very immature ones (arrow). Also note multinucleated forms and occasional plasma cells. $\times 550$.

amount of cytoplasm seen in these cells was homogenous and pink. Somewhat less common were cells having very similar nuclei, but with a relatively large amount of clear pink cytoplasm, which sometimes had a purplish tinge. A third type of cell which was observed only occasionally had an oval, or round nucleus, with medium coarse, basophilic blocking at the periphery, one or two nucleoli and a variable amount of basophilic cytoplasm. Special differential stains for hemoglobin failed to reveal its presence in any of these cell types. Occasional mitotic figures were seen. Scattered through these areas there were also a few reticulo-endothelial cells and erythrocytes. Although most of the bone marrow had the above histologic picture, there were also large open spaces in which were packed numerous erythrocytes and neutrophils together with the other cellular components of bone marrow. In these vascular areas, there could be found only scattered representatives of the above myeloma cells. It is thought probable that the sternal marrow had been aspirated from such a vascular area. True plasma cells were seen only rarely with the hematoxylin and eosin stain. In tissue treated by Giemsa stain, they seemed to be more common and cells, which would have been called erythroblastic myeloma cells with the hematoxylin and eosin stain, were seen to have a very faint cartwheel pattern.

DISCUSSION

The first report of an erythroblastic myeloma was published by Ribbert⁶ in 1904. A fairly complete review of 643 multiple myelomas of all types was published in 1937 by Atkinson.¹ He classified the cases he had reviewed as follows: not classified, 332; plasmacytoma, 207; lymphocytoma, 16; myeloblastoma, 27 (3 others doubtful); myelocytoma, 24; erythroblastoma, 5 (1 other doubtful); and mixed, 28.

Since then the total number of cases of multiple myeloma has been brought up to almost 1,000.² Two additional cases of erythroblastic myeloma were reported by von Keiser⁷ and by Noothoven van Goor.⁴ Von Keiser also mentioned the case of Schridde as being one of erythro-myeloblastoma and considered two cases reported by Harbitz and one by Schur and Loewy as possibly those of erythroblastoma.

A very unusual case diagnosed as hemoblastic sarcoma and said to be the first of its type was reported by Perla and Biller.⁵ They found an actively invasive and metastasizing tumor composed of islands of hemocytoblastic and erythroblastic cells in well organized hemopoietic nodules. Seventeen years previously, this patient had been successfully treated for polycythemia vera with x-rays and benzene.

The slides of the present case were studied at the National Institute of Health, Bethesda, Maryland, where it was believed that this was probably a case of erythroblastic myeloma. The histologic appearance of the myeloma cells is typical of the erythroid series. The fact that no hemoglobin was found in these cells can probably be attributed to their immature state. In view of the fact that plasma cells appeared to be more numerous in Giemsa-stained sections, it is possible that this is a case in which the plasma cells, originating from the

stem hemocytoblasts, have become differentiated toward the erythroid series. This phenomenon has been shown to occur in several cases with differentiation toward the myeloid series² and in one case toward a megakaryocytic myeloma.³ This case should therefore be classified as an erythroid multiple myeloma.

SUMMARY

A case is presented of multiple myeloma without Bence-Jones protein, in which the myeloma cells were histologically of the erythroid series.

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EDITORIAL

THE TUBERCULIN REACTION

The local tuberculin reaction as a specific diagnostic aid became of practical importance early in this century with the appearance of the Pirquet scarification test, the Moro percutaneous method, the Mendel-Mantoux intracutaneous test and the Calmette ophthalmo reaction. These tests were followed many years later by the Craig multiple puncture technic. On the basis of the reactions obtained with the Pirquet test particularly, and the use of full strength old tuberculin (O. T.) for this purpose, many erroneous conclusions were drawn, including the deduction that all adults had been infected with tuberculosis in childhood. This latter conclusion was the obvious result of the use of high nonspecific concentrations of old tuberculin which was brought in contact with the deeper tissues by scarification. Although yielding positive results in certain instances, the Moro inunction test failed in most cases because the tuberculin was fundamentally water-soluble and not oil-soluble. The ophthalmo test proved dangerous, and the only test to survive was the Mendel-Mantoux intracutaneous method which, of necessity, used graded dilutions of tuberculin. Furculow *et al.* (Pub. Health Reports, 56: 1082-1100, 1941) and Dash *et al.* (Am. Rev. Tuberc., 54: 159-165, 1946) have pointed out that insistence on the use of relatively high nonspecific concentrations of tuberculin in this latter test to match high percentages of reactions obtained among noninfected persons in various surveys constitutes a useless abuse of a valuable method. There were, however, several drawbacks to the Mendel-Mantoux injection method, *viz.*, it required aseptic implements, it was accompanied by a certain mental anguish precluding repeated tests, and its use had to be guarded in the tuberculous. The crying demand for a simpler, less objectionable procedure led to the introduction of a number of substitute dermal methods. Among these methods was the scientifically objectionable and rather unsatisfactory patch test (Am. J. Dis. Child., 54: 1019-1024, 1937), employing adhesive tape as a means of application, which was devised owing to lack of ingenuity and because a suitable tuberculin for transdermal application was not available.

In 1943, the process by which tubercle bacilli produced tuberculin was discovered, and autolytic tuberculin, a pure and bacteriologically clean tuberculin, was described (Am. Rev. Tuberc., 48: 443-452, 1943). This substance was hygroscopic when dry (Tuberculology, 8: 72-77, 1946) and could be appropriately suspended in the form of a fine powder in a quickly evaporating non-watery solvent of suitable composition. Thus, it acted when applied to the skin as a nonirritating adhesive. Furthermore, the dry tuberculin could be properly kept in suspension and free from water until it had been applied to the skin, when it became rapidly available in soluble form for the specific reaction to occur if the tuberculo-allergic condition was present. In comparative tests the results of the transcutaneous tuberculin test with autolytic tuberculin equal those obtained by the Mendel-Mantoux method with specific amounts of pure

tuberculin used intracutaneously. The transcutaneous method, however, can be used on the tuberculous as well as nontuberculous person without the danger of producing severe detrimental local or focal reactions of serious import such as are inherent in the intracutaneous method. Basically, the transdermal autolytic tuberculin test can be applied repeatedly and as easily as finger nail polish, and this tuberculin does not elicit nonspecific reactions in normal individuals and, therefore, does not require a control application. This simple and valuable diagnostic aid should broaden the scope of diagnosis of tuberculosis, and its use by the physician in any field where tuberculosis is a problem is potentially unlimited.

In an editorial in 1946, Hilleboe of the U. S. Public Health Service (Pub. Health Reports, 61: 1561-1563, 1946) stated, "Because it is basic in the control of tuberculosis, the importance of the tuberculin test should have first consideration. It must be clearly understood at the outset that mass radiography has not displaced the tuberculin test."

H. J. CORPER

BOOK REVIEWS

Autopsy Diagnosis and Technic. Ed. 2. By OTTO SAPHIR, M.D., pathologist, Michael Reese Hospital; professor of Pathology, University of Illinois Medical School, Chicago. 405 pp., 69 illus., 18 tables. \$5.00. New York: Paul B. Hoeber Company, 1946.

In the second edition of this excellent and concise manual, the chapter on the breast has been expanded, and other chapters have been added describing the findings in vitamin deficiencies and in certain tropical diseases.

This book both describes the technic of the performance of the autopsy and furnishes an excellent description of the gross pathologic lesions likely to be encountered. A differential diagnosis is furnished for many of the conditions. Also of special value is a chapter on securing of the autopsy permit and the medico-legal aspects of the autopsy. Special procedures to be followed in the performance of autopsies on the stillborn and on the newborn are described. Clear illustrations accompany the text.

The procedures which have been described are, in general, those which are usually used in most institutions. It is to be remembered, however, that the exact technic in any given instance will depend upon individual circumstances. Only one method of removal of organs has been described, but there are other methods that may be preferred by the prospector. Likewise, in the opening of the heart, methods other than that outlined have been found equally satisfactory. In instances of probable recent occlusion of a coronary artery, it is preferable to cut the artery by multiple cross-sections rather than to open it along its length with a scissors.

This book should prove of continued value, not only to those who are beginners in the performance of autopsies, but also to more experienced pathologists.

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WALTER A. STRYKER

Diseases of the Retina. By HERMAN ELWIN, M.D., Senior Assistant Surgeon, New York Eye and Ear Infirmary. 593 pp., 170 illus., 19 in color. \$10.00. Philadelphia: The Blakiston Company, 1946.

This book is a compend of current writings in the fields of pathology and therapeutics. No claim is made for originality. The selection of material, its order, and emphasis are the authors. The arrangement is in 8 parts, under the following headings:

1. Diseases of Retina Resulting from Disturbances in Circulation
2. Diseases of Retina Resulting from Vascular Malformations
3. Degenerative Diseases of Retina on a Hereditary Basis
4. Inflammatory Diseases of Retina
5. Tumors of Retina
6. Diseases of Retina Leading to Retinal Detachment
7. Developmental Anomalies of Retina
8. Radiation Injuries of Retina

The definite meanings applied in pathology to such terms as degeneration, inflammation, circulatory disturbances, and malformations are not strictly adhered to in the book. As is often the case, the emphasis placed upon some disease processes is disproportionate. A number of the commoner retinal lesions such as retinal pathology of glaucoma, traumatic lesions, foreign bodies, and retinic atrophy, suffer from neglect. A section on hyaline bodies of the lamina vitrea relates to the choroid and not the retina.

Obviously, the book was written for the busy clinician and may well serve as a clinical reference. While the book has many merits, one criticizes it only because it could have been done even better.

The table of contents and the index are helpful.
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PARKER HEATH

OBITUARIES

RICHARD WILLIAMS CRAGG

Richard Williams Cragg of Vallejo, California, died February 18, 1946, of coronary occlusion, while serving as chief pathologist at the Naval Hospital at Mare Island, California. Dr. Cragg, who was born in Cincinnati in 1905, received his medical degree from the University of Cincinnati College of Medicine in 1931. In October 1932, he joined the Mayo Foundation, Rochester, Minnesota, as a fellow in pathology. He was later appointed first assistant in pathologic anatomy and surgical pathology, consulting physician in pathologic anatomy at the Mayo Clinic, instructor in pathology at the foundation and assistant professor of pathology. He began active duty as a first lieutenant in the medical corps of the U. S. Naval Reserve on July 1, 1942 and was promoted to lieutenant commander before his death. Dr. Cragg became a member of the American Society of Clinical Pathologists in 1937.

FRIEDRICH ALEXANDER HECKER

Friedrich Alexander Hecker, of Ottumwa, Iowa, died on June 3, 1945. He received his medical degree from Kansas School of Medicine, Kansas City, in 1913. He was a specialist certified by the American Board of Internal Medicine and served as pathologist at St. Joseph Hospital. He was a member of the Society of American Bacteriologists, a fellow of the American College of Physicians and, since 1925, a member of the American Society of Clinical Pathologists.

TECHNICAL SECTION

DETERMINATION OF Rh-ANTIBODIES AND BLOOD GROUPING BY THE CAPILLARY METHOD*

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At the present time, relatively few laboratories perform tests for Rh antibodies. As practicing physicians become increasingly aware of the real and potential complications of Rh incompatibility, there will be a greater demand for such tests. It has recently been estimated that 90 per cent of hemolytic transfusion reactions which are not due to errors in A-B-O grouping are caused by Rh incompatibilities.⁷ A new group of potential victims of this condition also has been created by the recent war. The emergency administration of group O blood to battlefield casualties at a time when it was not expedient to perform preliminary Rh testing made it inevitable that many Rh-negative recipients should receive Rh-positive blood and thereby become sensitized. Examination of the serum of 110 Rh-negative individuals who received group O blood during combat revealed the presence of Rh antibodies in 40 per cent.² These persons have not been harmed so long as they receive no additional transfusions with Rh-positive blood. The study of Rh-negative women bearing Rh-positive fetuses has also verified the value of anti-Rh determinations. In some cases, pre-term delivery by cesarian section has aided in preserving the life of the infant.

Two methods of Rh antibody determination are commonly used at the present time. They are the slide test of Diamond and Abelson³ and the tube or conglutination test of Wiener.⁹ These methods are of unquestioned value, but our experience has been that they require the services of a technician who has had considerable experience in performing and reading the tests. Equivocal results are sometimes obtained even when extreme care is exercised in carrying out the procedure. Personal communications with directors of other laboratories in which the same tests are used indicate that they also have had difficulty in interpreting results. The open-slide test is especially subject to non-specific agglutination and rouleaux formation.⁵

The method for Rh antibody determination herein described is an adaptation of the capillary test described by Chown in 1944¹ for Rh testing. In his survey of present methods of Rh antibody determination, Diamond refers briefly to the capillary method, but dismisses it as being too difficult to read.⁵ Our

* Received for publication, September 23, 1946. The opinions expressed are those of the author and are not to be construed as necessarily reflecting the views of the Navy Department.

experience suggests that its greatest advantage over other methods is the clarity of the readings and ease with which an uninitiated observer may learn to differentiate a positive from a negative result. It is rapid and economical and requires a minimum of equipment. No rotator or centrifuge is used and there is no glassware to clean. Results may be checked with a microscope, or recorded photographically, and the appearance of rouleaux is rarely encountered.

In the past, it has been difficult to demonstrate Rh antibodies in some women with strong clinical evidence of Rh sensitization. This has been ascribed to the presence in their serum of unusual antibodies, which have been variously termed "inhibiting," "incomplete," "blocking,"⁴ or "univalent"¹⁰ antibodies. They differ from the usual agglutinins in that no agglutination is obtained *in vitro* in the presence of saline or other crystalloid solution. In former tests,



FIG. 1. Materials required for Rh testing. From left to right are: (a) test tube containing Rh typing serum, (b) unknown cell suspension, (c) petrolatum, (d) capillary tubes, (e) hand-lens. For A-B-O- grouping, merely substitute A anti-B and B anti-A grouping serums for the Rh typing serum. For Rh antibody tests, place the unknown serum in the first test tube and Rh-positive group O test cells in the second tube.

the use of saline as a diluent for the unknown serum and as a medium for suspending the test cells has prevented agglutination *in vitro* and has led to confusion. The substitution of inactivated AB serum, human serum albumin, compatible inactivated serum, or plasma for the saline permits detection of agglutination in the presence of blocking antibodies. This is the basis for the test which Wiener has designated the "conglutination test." The capillary method which follows offers the same advantages as the conglutination test, since it employs no saline.

MATERIALS

1. Capillary tubes, inner diameter about 0.5 mm., and length from 10 to 15 cm. These may be procured from laboratory supply houses.

2. Rh typing serum, for checking the test cell suspension.
3. A-B grouping serum or grouping globulin, for checking the test cell suspension.
4. Normal serum albumin (human), prepared for intravenous use,⁶ preferably without preservative, since Rh antibodies are destroyed by merthiolate.⁸ This is used as a diluent for unknown serum when titer is made.
5. Normal group A-B serum, inactivated at 56 C. for thirty minutes.
6. Petrolatum, kept in a bottle cap for ease in handling.
7. Plastic rack, measuring 40 by 5 by 5 cm., with about 30 notches, placed so that tubes are held at an angle of 45 degrees. These may soon be available from laboratory supply houses.
8. Hand magnifying lens.

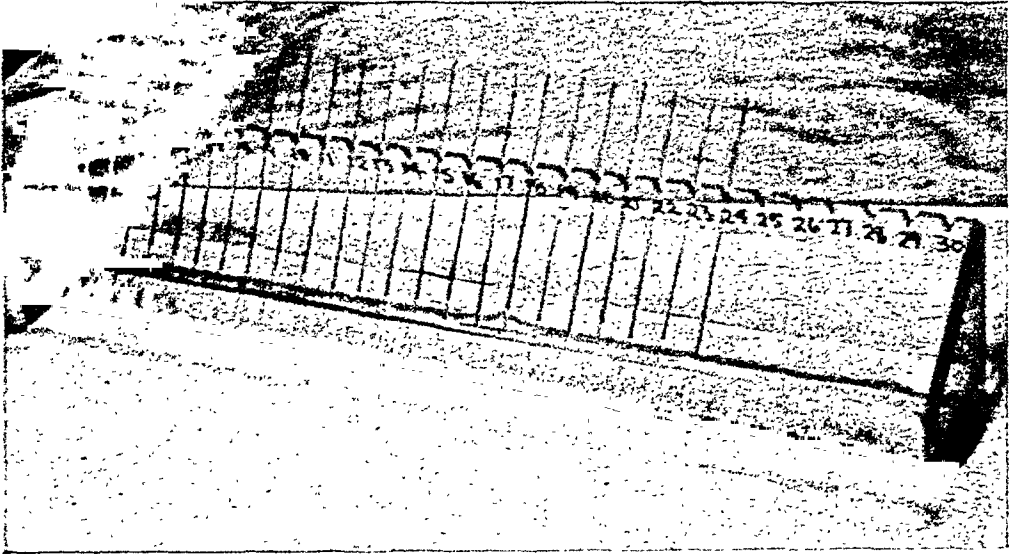


FIG. 2. PLASTIC RACK WITH CAPILLARY TUBES IN PLACE

METHOD

1. Prepare test cell suspension. This may consist of a 10 per cent to 20 per cent suspension of erythrocytes from one individual or a pool of erythrocytes from several Rh-positive group O individuals. It is preferable to pool the cells of three to six persons as this gives reasonable assurance that all subgroups of Rh (Rh^c, Rh', and Rh'') will be represented. The suspension is made by first allowing the blood to clot and contract in a test tube and then pouring off most of the supernatant serum. Then next, the tube is shaken so that enough cells are freed from the clot to give the proper suspension in the remaining serum. No further preparation is necessary. Clots from blood specimens submitted to the laboratory for blood chemistry or routine serology may be used.
2. Separate serum from the blood of the patient to be tested and inactivate it at 57 C. for thirty minutes.
3. Touch the surface of the unknown serum with a capillary tube and allow the fluid to rise about 2 cm. Remove the capillary and wipe the outside with gauze.

4. Dip the capillary into the test cell suspension and allow an equal, or slightly greater, volume of fluid to rise in the tube. It is usually necessary to tilt the tube toward the horizontal at this point in order for the cell suspension to enter the capillary.

5. Wipe the tube again and tilt it back and forth until the cell suspension and unknown serum have mixed. Three or four times usually will suffice.

6. Dip the capillary into the petrolatum so that the lower end becomes sealed; place it on the rack and incubate for thirty minutes at 37 C.

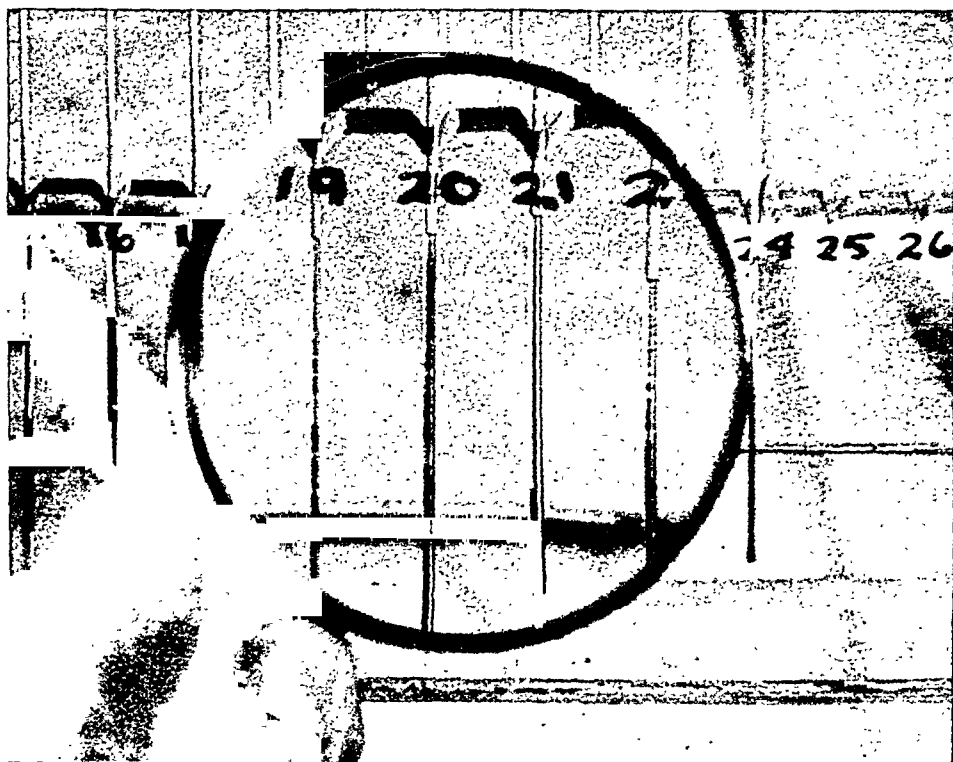


FIG. 3. RESULTS OF TESTS

Agglutination is visible in tubes 19, 20 and 22. Tube 21 contains a fine line of sedimented cells and is negative.

7. Readings are made against a bright light and a white background. Positives are indicated by coarse agglutination and negatives by a smooth line of sedimented cells extending the entire length of the column of fluid in the tube. A hand lens is helpful, but not essential, for accurate readings.

8. Serums which give agglutination on the direct test may be titered by making serial dilutions with A-B serum or serum albumin.

DISCUSSION

This method utilizes the gravitational force to provide very gentle agitation of the erythrocytes as they settle in the capillary tube. Since the tube rests at a 45 degree angle, the cells tend to "snowball" and to adhere to the walls even when agglutination is weak. In other methods it is difficult to provide sufficient

agitation to allow the cells to clump without disrupting the agglutination which is very fragile.

Thirty-four serums suspected of containing Rh antibodies have been examined by the slide test, the conglutination test and the capillary test with results which are shown in Table 1. In all instances, negative controls were employed, consisting of (a) a test cell suspension of Rh-negative group O cells plus unknown serum and (b) known negative serum (containing no Rh antibodies) plus the pooled Rh-positive group O cells.

TABLE 1

COMPARISON OF RESULTS OF THREE METHODS OF Rh ANTIBODY DETERMINATION WITH 34 SPECIMENS FROM 24 PATIENTS SUSPECTED OF Rh SENSITIZATION

	UNKNOWN SERUM PLUS POOLED Rh-POSITIVE CELLS		
	Positive	Doubtful	Negative
Slide test.....	25	0	9
Conglutination test.....	25	2	7
Capillary test.....	27	0	7

TABLE 2

PROTOCOL FOR Rh ANTIBODY TESTING

1. Pooled test cells plus A anti-B serum.
2. Pooled test cells plus B anti-A serum.
3. Pooled test cells plus anti-Rh typing serum.
4. Pooled test cells plus A-B serum (negative control).
5. Pooled test cells plus positive control.
6. Rh-negative group O cells plus unknown serum.
7. Rh-negative group O cells plus anti-Rh typing serum.
8. Pooled test cells plus unknown serum.
9. Pooled test cells plus unknown serum, diluted 1:2.
10. Pooled test cells plus unknown serum, diluted 1:4.
11. Pooled test cells plus unknown serum, diluted 1:8.
12. Pooled test cells plus unknown serum, diluted 1:16.
13. Pooled test cells plus unknown serum, diluted 1:32, etc.

The results shown in Table 1 were confirmed by 133 additional capillary tests performed on the 27 positive serums and by 50 tests with normal serums chosen at random as negative controls.

Wiener stated that the conglutination test may have greater sensitivity for A-B-O grouping than the usual method using an isotonic saline cell suspension.⁹ Since the capillary method is in reality a modification of the conglutination test, it has been tried for determination of A-B-O grouping. It was found that standard A anti-B and B anti-A grouping serums diluted 1:5 with serum albumin or human A-B serum produced excellent results. The mechanics of the test are identical with the technic described above for Rh antibody determination except that A-B grouping globulin or serum is mixed with a suspension of unknown cells. The cell suspension is prepared from a clot in the same manner as the

Rh-positive test cell suspension for Rh antibody determination. An extensive series of A-B-O groupings has not yet been completed but several hundred preliminary trials have proved satisfactory and further investigation is warranted.

Rh typing as described by Chown can be done with great facility. The only difficulty which may be encountered is in testing of Rh subgroups, where the typing serum for Rh' and Rh'' may have been prepared by adding Rh^o blocking serum to the Rh' or Rh'' serum. The omission of saline in this test will allow false agglutination because of the Rh^o present and will not be a true test for Rh' or Rh''. This is avoided when subgroup typing serum containing only anti-Rh' or anti-Rh'' is employed. Of the last 272 specimens of blood tested in this laboratory, 236 (86.7 per cent) were Rh-positive.

Since Rh testing, A-B-O grouping, and Rh antibody determination may all be done with the same method, it has proved expedient to use the protocol shown in Table 2 in determining Rh antibodies. All of the pre-testing and controls may be set up on the same rack.

SUMMARY

A modification of the capillary technic of Chown is presented which provides an efficient method of determining Rh sensitization, Rh type, A-B-O groups and other factors such as M, N and P. It requires simple equipment and gives fewer equivocal results than either the slide test for Rh antibodies or the conglutination test. Test cells for Rh antibody testing are suspended in their own serum and give more consistent results when pooled. Further study may indicate that this method is more sensitive than previous ones. The possibility of interference of atypical hemagglutinins in routine A-B-O grouping as it is now done, using saline solution, should be investigated. The capillary test is ideally suited for doing a large series of conglutination tests.

Acknowledgment. The author wishes to express his gratitude for the excellent technical assistance provided by Harold R. Langbehn, Chief Pharmacist's Mate, U. S. Navy.

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SIMPLIFIED DETERMINATION OF BLOOD VOLUME*

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Many methods of blood volume determination have been described in the literature.^{2, 5, 8, 9, 11, 12} The purpose of this paper is to present results using a method similar to that reported by Gregersen⁵ which has been found to be accurate, relatively simple, and which may be carried out with available clinical laboratory equipment. The range of clinically normal values for blood and plasma is quite broad^{1, 3, 5, 8, 13, 14} and, from the data presented, it will be noted that the results obtained consistently fall within this range.

The procedure consists of the injection of a known amount of the dye, T-1824 (Evans Blue) and the calculation of blood volume from the dilution.

EQUIPMENT

Three 10 ml. syringes (one standardized, see "Methods")
Two 18-gauge needles for venipuncture
Two 4 ml. 100 mm. by 8 mm. hematocrit tubes
One ampule heparin, 10 mg./ml.
Sterile solution of T-1824 (Evans Blue)
Coleman Junior Spectrophotometer model 6 with 10 by 75 mm. cuvettes

METHODS

Preparation of Solution of Dye

The solution of the dye T-1824 is prepared by dissolving 2 gm. in 500 ml. distilled water. It is passed through a 3-G-3 Jena sintered glass filter and divided between a suitable number of brown bottles and autoclaved. Following this, rubber diaphragm stoppers are inserted and the bottles stored in the dark. Such solutions remain stable and sterile indefinitely when used with reasonable care.

Determination of K Value

To find the K value, it is necessary to make dilutions of the dye and determine their densities with the spectrophotometer at a wave-length of 620 millimicrons, using 10 by 75 mm. cuvettes. This is done as follows:

1. One ml. of dye solution is delivered into a 50 ml. volumetric flask and made up to volume with distilled water.
2. One-half ml. of dye solution is delivered into a 50 ml. volumetric flask and made up to volume with distilled water.

* This study was supported in part by a grant from the Wisconsin Alumni Research Foundation. Received for publication, December 7, 1946.

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3. From each of these flasks, 0.2 ml. is delivered into separate cuvettes and 1.8 ml. of plasma or serum added. This gives concentrations of 0.008 and 0.004 mg. per ml., respectively.

4. The optical densities of the dye-containing samples are determined with the spectrophotometer, using a control made up of 0.2 ml. of 0.9 per cent saline and 1.8 ml. serum or plasma.

5. The formula $K = \frac{D}{C}$, where D is density and C the concentration, is used

in the computation of the K value.

A good quality 10 ml. syringe should be calibrated, either by the weight of a delivered volume of distilled water, or by filling with standard acid and titrating a delivered volume with a standard base. It is necessary to make a mark on the plunger and on the barrel beyond the 10 ml. mark, so that when they are opposed, the end of the plunger will be at the 10 ml. level, since the color of the dye solution is so intense that it obscures the tip of the plunger.

Preparation of the patient consists of withholding fluid and restricting activity for eight hours prior to the test.

After the necessary equipment has been sterilized and thoroughly dried, two hematocrit tubes are placed in a rack. One drop (0.06 cc.) of heparin is placed in each tube. Care must be taken that the tubes are chemically clean and that the heparin is not deposited along the rim of the mouth. If the tubes are not absolutely clean, some hemolysis may result. If heparin is deposited at the mouth, it may be covered by the cork stopper that is to be used. The amount of dye to be injected should be approximately 0.3 mg./kg. of body weight. The calibrated syringe is filled with the correct quantity of dye. With the patient in a reclining position, 5 ml. of blood is removed from a cubital vein with a dry syringe. It is important that there be no stasis of venous blood. Consequently, the tourniquet must be removed at least one minute before the blood is drawn into the syringe. The measured amount of dye is then injected slowly through the same needle, the syringe being filled and emptied at least five times with the patient's blood in order to wash out all traces of dye. A notation of the time must be made at the beginning of the injection of the dye. While the dye is being injected, an assistant should take the freshly drawn blood and fill the first hematocrit tube to the 10 cm. mark. This should be done quickly, but with caution, in order that there be no frothing which may cause hemolysis. The cork stopper should be inserted and the tube gently inverted several times until good mixing with the heparin is effected.

Approximately nine minutes after beginning the injection of the dye, a clean needle on a clean dry syringe is inserted into a cubital vein. The tourniquet is removed and time allowed for venous stasis to correct itself, and when exactly ten minutes have elapsed from the time of injection of dye, 5 ml. of blood is again withdrawn. The needle and syringe are removed and the blood cautiously placed in the second hematocrit tube which is inverted and stoppered in the same manner as before. It will be noted that some blood will collect at the junc-

tion of the stoppers and the tubes. This should be carefully removed with a cotton-tipped applicator, the corks likewise wiped off and then replaced. Both tubes should then be centrifuged for thirty minutes at 3000 R.P.M.

The hematocrit is then read in the conventional manner. The supernatant plasma is removed by means of capillary pipets, placed into 10 by 75 mm. cuvettes and centrifuged to remove any suspended cells. The first cuvette which contains the sample without dye is placed in the spectrophotometer with the wave length set at 620 millimicrons. The machine is adjusted to 0 density with this sample. The second sample which contains dye is then inserted and the density read and recorded. The plasma volume (PV) may now be calculated

with the use of the formula $PV = \frac{MK}{D}$, where M is number of mg. of dye injected,

K is the constant as previously described, and D is the density reading of the spectrophotometer.

Total blood volume (TBV) is calculated from the formula $TBV = \frac{PV}{P} \times 100$,

where PV is the plasma volume from the previous calculation and P is the corrected volume of plasma per 100 ml. The corrected volume of plasma is derived from the formula,

$$P = 100 - \left[\frac{\text{volume of cells}}{\text{volume of plasma and cells}} \times 100 \right]$$

RESULTS

The accompanying table shows the results of 30 determinations on male patients, the determination on each of five patients having been repeated the following day. It will be noted that the values fall within the range of normals as compiled by Noble and Gregerson.¹¹ In every instance, the method was checked against that of Gibson and Evans² in which serum is used instead of plasma. For reasons as yet unknown, the plasma values have averaged 5 per cent higher than those of the serum. This is apparently not due to the use of liquid heparin as an anticoagulant. Samples of blood from the ten normal adult males were divided so that one portion was allowed to clot in a paraffin-lined tube, and the other portion was placed in the usual hematocrit tube with 0.06 ml. heparin. Equal quantities of dye were then added to the samples of plasma and serum and mixed. The samples of plasma and serum showed no difference in optical density.

While extrapolation to zero time by employing a series of successive samples would in all probability give more accurate values, it was found that in order to obtain a reliable straight line too many samples were required to make the method practical. Consequently a single ten minute sample was used. The validity of using a single sample has been reported by Gregersen,⁵ Noble and Gregersen,¹¹ Miller,¹⁰ Hopper, Tabor and Winkler,⁶ Hopper, Winkler and Elk-

inton,⁷ Gilder, Muller and Phillips⁴ have shown that there is complete mixing of the dye with the circulating plasma in this period of time. The fact that the

TABLE 1

COMPARISON OF PLASMA AND TOTAL BLOOD VOLUMES USING SERUM AND PLASMA SAMPLES

CASE NUMBER	PLASMA VOLUME CALCULATED FROM								BLOOD VOLUME CALCULATED FROM					
	Hematocrit	Surface area sq. M.	Plasma sample ml.	Serum sample ml.	Plasma sample ml. per Kg.	Serum sample ml. per Kg.	Plasma sample ml. per sq. M.	Serum sample ml. per sq. M.	Plasma sample ml.	Serum sample ml.	Plasma sample ml. per Kg.	Serum sample ml. per Kg.	Plasma sample ml. per sq. M.	Serum sample ml. per sq. M.
1	42.7	1.76	2645	2431	41.3	37.9	1502	1324	4614	4242	72.1	66.3	3621	3410
2	45.7	1.64	1915	1860	34.5	33.5	1167	1134	3526	3239	63.5	58.3	2150	1975
3	50.3	1.64	2006	1816	32.6	29.6	1223	1107	4036	3653	65.8	59.5	2461	2227
4	44.1	1.67	2568	2537	43.5	43.0	1538	1519	4593	4538	77.8	76.9	2750	2717
5	43.1	1.95	3110	3028	40.2	39.2	1595	1553	5465	5321	70.8	68.9	2802	2728
6	49.0	1.90	2469	2431	32.3	31.8	1299	1279	4841	4766	62.6	61.6	2548	2508
7	53.7	1.74	2168	2111	33.6	32.7	1246	1213	4682	4553	72.5	70.5	2691	2616
8	47.2	1.70	2615	2469	43.6	41.1	1538	1452	4952	4675	82.4	77.9	2913	2750
9	45.8	1.83	2587	2469	39.8	37.9	1413	1349	4741	4555	72.9	70.0	2591	2489
10	52.8	1.84	2279	2198	31.9	30.8	1238	1194	4829	4656	67.7	65.2	2624	2530
11	47.8	2.00	3110	2939	37.0	34.9	1555	1469	5957	5611	71.8	66.8	2978	2805
12	49.2	1.84	2862	2534	41.4	36.7	1555	1377	5633	4988	81.6	72.3	3055	2711
13	52.2	1.85	3124	2961	45.2	42.9	1687	1600	6535	6192	94.7	89.7	3532	3347
14	44.0	1.88	2926	2701	43.7	40.2	1556	1437	5225	4820	77.9	71.9	2779	2564
15	47.0	1.63	2481	2400	41.3	40.0	1522	1472	4681	4528	78.0	75.4	2871	2777
16	55.2	1.85	3068	2862	42.7	39.8	1658	1547	6459	6388	89.9	88.9	3491	3453
17	47.1	1.75	2815	2555	40.5	36.7	1608	1460	5304	4829	76.3	69.9	3031	2759
18	48.2	1.85	2783	2632	36.9	35.9	1504	1422	5372	5081	71.2	67.3	2904	2746
19	45.8	2.06	3544	3424	44.6	43.1	1720	1662	6538	6317	82.3	79.5	3173	3066
20	45.6	1.94	3508	3479	47.4	47.0	1808	1793	6448	6376	87.2	86.1	3323	3285
21-A	46.3	1.75	3253	3106	50.0	47.7	1859	1775	6057	5783	93.1	88.9	3461	3306
21-B	45.5	1.75	3293	3084	50.6	47.4	1881	1762	6042	5658	92.9	87.0	3452	3233
22-A	49.7	1.72	3057	2877	48.0	45.2	1777	1672	6077	5719	95.7	90.0	3475	3262
22-B	49.9	1.72	2951	2887	46.4	45.4	1716	1678	5890	5762	92.7	90.7	3424	3350
23-A	43.4	1.65	3130	2948	51.0	48.1	1897	1787	5530	5208	90.2	84.9	3351	3156
23-B	43.5	1.65	3101	3057	50.5	49.8	1879	1852	5488	5410	83.0	81.7	3326	3280
24-A	41.5	1.90	3522	3324	50.3	47.5	1874	1849	6020	5682	86.0	81.1	3168	2990
24-B	42.1	1.90	3551	3390	48.4	50.7	3168	2990	6132	5854	87.6	83.5	1868	1768
25-A	47.8	1.82	3122	3014	48.0	46.3	1715	1106	5980	5773	92.0	88.8	3286	3172
25-B	42.8	1.82	3337	3124	51.3	48.0	1833	1716	5833	5461	89.7	84.0	3205	3000
Average..	43.3	1.80	2930	2754	42.6	41.0	1651	1555	5499	5251	80.7	76.6	3010	2866

Cases marked A and B represent the same patient.

All determinations on samples of serum were done according to the method of Gibson and Evans.¹

average value in the present paper, as seen from the tabulated data, falls so close to those reported by other investigators further confirms the validity of such a procedure.

COMMENTS

This procedure may be adapted to other types of photo-electric instruments provided that the power source is constant, a suitable light filter is used, small cuvettes are employed, and that the optical system permits the passage of sufficient light without undue diffusion.

The value of blood volume determinations in many clinical conditions such as shock, polycythemia vera, hyperthyroidism, myxedema, obesity, or for the determination of total circulating protein is well established. The availability of a simple and reasonably accurate method is thus desirable in view of the fact that such conditions are not infrequently encountered in a general hospital population.

SUMMARY

A simplified procedure for blood volume determination has been studied and shown to give consistent and reproducible results. A comparison of plasma volume values using samples of plasma and serum revealed the former to give values 5 per cent higher than the latter.

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ZINC IN HUMAN TISSUES

DETECTION AND DETERMINATION BY DITHIZONE*

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The detection of zinc in biologic tissues has, at best, always been tedious and troublesome. In the usual qualitative chemical scheme of analysis, zinc is detected by precipitating it as the white sulfide. When analyzing human tissues, the zinc sulfide obtained is invariably colored brown to black. Successive reprecipitations do not entirely eliminate all traces of these colored contaminants and, thus, it is well-nigh impossible to obtain a pure white zinc sulfide precipitate.

The object of the present research was to test the feasibility of completely separating the zinc from all other cations and contaminants by extracting the aqueous phase with a chloroform solution of dithizone. If this extraction were successful, the zinc dithizone complex in the chloroform phase could then be decomposed by dilute acid and the zinc re-extracted from the organic solvent by shaking it with water. The acidity of this aqueous solution could then be adjusted for the precipitation of the pure zinc sulfide.

Dithizone is an organic dye soluble in chloroform, yielding a green solution. Many cations, silver, mercury, lead, tin (stannous), bismuth, cadmium, copper, cobalt, nickel, zinc, manganese (manganous), produce colored complexes (orange, red or violet) with the dye, which are insoluble in water but are soluble in chloroform. Hence, if a neutral or slightly alkaline solution of any one of the above cations is shaken with a solution of the dye, the metal complex will pass into the chloroform layer. The latter can be drawn off and the extraction repeated until all of the metal has been removed. When the extractions are done quantitatively, with a standard dithizone solution, the process is known as "extractive titration."

In the usual course of an analysis, several of the extractable cations may be present. The problem is then to extract the desired cation, and at the same time prevent the extraction of the other cations. This is done (a) by adjusting the aqueous solution so that it has the proper pH for the extraction of desired cation and (b) by adding reagents, such as cyanide or thiosulfate, which form complexes with the other cations and thus prevent them from reacting with dithizone.

Fischer and Leopoldi,⁴ Sandell,⁶ Holland and Ritchie,⁵ Cowling and Miller,³ and Alexander and Taylor¹ have described methods for the detection and determination of small quantities of zinc based upon the above principles. These methods are more or less tedious and time-consuming, because they require several extractions. It was our purpose to devise a simple and direct method for the detection and determination of small quantities of zinc in biologic material.

* Received for publication, September 6, 1946.

EXPERIMENTAL

Experiments were performed to discover under just what conditions zinc ions, to the exclusion of all of the above-mentioned cations, will be extracted by a chloroform solution of dithizone. It was found that in an aqueous solution having a pH of 5.5 ± 0.2 and containing sodium potassium tartrate, sodium thiosulfate, potassium cyanide and sodium acetate, only zinc, cadmium and cobalt (the latter two only if more than 300 micrograms are present) will be extracted by the dithizone solution. Contamination of the extracted zinc by cobalt was not considered important because the latter is not likely to occur in appreciable quantities in human organs. Cadmium is, therefore, the only cation which may be extracted with the zinc, and which must subsequently be removed if present. It was also found that the quantity of iron which may be present in blood and tissues, 3 mg. in 5 gm. has no oxidative effect upon the dithizone. Based upon the above facts, the following method was devised and elaborated.

METHOD

Reagents Required (all C.P. grade):

- Nitric acid, concentrated
- Sulfuric acid, concentrated
- Digestion mixture: composed of two volumes of 66 per cent perchloric acid and one volume of concentrated nitric acid
- Ammonia, concentrated solution
- Hydrochloric acid, concentrated
- Acid meta cresol purple indicator, 1 per cent solution
- Hydrogen sulfide, tank or generator
- Sodium potassium tartrate solution, 20 per cent
- Methyl red indicator, 1 per cent solution
- Litmus paper
- Buffer solution: 550 gm. of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$), 10 gm. of potassium cyanide (KCN), and 90 gm. of sodium acetate ($\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$) are dissolved in one liter of warm water. Dilute acetic acid (1 to 4) is added until the solution had a pH of 5.5. The solution is then diluted to two liters.
- Dithizone Solution* (standardized): 0.04 gm. of dithizone is dissolved in one liter of chloroform. The solution is filtered and allowed to stand for several hours. The dithizone solution is standardized against a standard zinc solution on the day it is to be used. The zinc content of the standard should be of the same order of magnitude as that of the solution to be analyzed. Should the latter have a much higher zinc content than the zinc standard, it is advisable to take a smaller aliquot of the solution of the tissue digest. The procedure for standardizing the dithizone is the same as that to be described in the method to follow.
- Standard zinc solution*: 63.5 mg. of zinc (metal) are dissolved in (1 to 1) nitric acid and 5 ml. of concentrated sulfuric acid are added. The solution is heated

until copious fumes of sulfur trioxide evolve. When cool, it is made up to exactly 500.0 ml. with distilled water (1 ml. = 0.127 mg. zinc).

PROCEDURE

Twenty-five gm. of finely ground tissue or of blood is placed into a 250 ml. Erlenmeyer flask, and 50 ml. of concentrated nitric acid is added. After allowing the mixture to stand in a warm place, for one hour, with occasional stirring, the flask and contents are placed on a steam bath and allowed to remain until all of the tissue is dissolved and the evolution of nitric oxide (NO_2) has virtually ceased. The contents are then permitted to cool to room temperature. In order to avoid explosive reactions during the perchloric acid digestion, any solidified fat is filtered off, through glass wool, and discarded. The filtrate is collected in a 300 ml. Kjeldahl flask.

When analyzing urine samples, at least 100 ml. should be employed. The sample is first evaporated to a syrupy consistency on the steam bath and 100 ml. of concentrated nitric acid is then carefully and gradually added, since a violent reaction ensues. The contents are permitted to remain on the steam bath until a clear yellow solution results, which is then transferred to a 300 ml. Kjeldahl flask.

The nitric acid digest of tissue, blood, or urine in the Kjeldahl flask, after adding 6 ml. of concentrated sulfuric acid and several small glass beads, is boiled over a free flame. When all of the nitric acid has been boiled off, charring begins and this is generally accompanied by frothing. It is well to lower the flame at this point, and then to increase it again when the charred material has ceased frothing. If during the charring the contents solidify, cool and add 2 ml. more of concentrated sulfuric acid and continue heating. When frothing has ceased, the flame is raised and the digestion mixture is added dropwise. After 0.5 ml. of the digestion mixture has been added, further addition is deferred until frothing again ceases. Additional 0.5 ml. portions are thus added dropwise until the hot liquid has changed in color from black to yellow or has become colorless. About 5 ml. of the digestion mixture is needed for 25 gm. of tissue and, if added during an interval of approximately ten minutes, will give the desired result. The heating is now continued until sulfur trioxide fumes are evolved. The flask and contents are then allowed to cool to room temperature. Ten ml. of water is added, and the contents are again boiled until sulfur trioxide fumes are evident. This treatment is repeated once more in order to insure the destruction and expulsion of all traces of nitric acid and oxides of nitrogen. The contents are then allowed to cool to room temperature, and the volume is made up to exactly 50.0 ml. by the addition of distilled water. The diluted digest is filtered, if necessary, and used for the detection and estimation of zinc.

A 10 ml. aliquot of the diluted digest is made alkaline to acid meta cresol purple with concentrated ammonia, diluted to 50 ml., and then made slightly acid with concentrated hydrochloric acid. The solution is heated to about 70 C. and hydrogen sulfide passed through for about five minutes. The flask is stoppered, and allowed to stand one-half hour. It is then filtered, the precip-

itate washed with two small portions of water, and the washings are added to the main filtrate. The precipitate is discarded. The filtrate is boiled to expel all of the excess hydrogen sulfide and to reduce the volume to about 10 ml. If, as is usually the case, cadmium is not present in the tissue, this hydrogen sulfide treatment may be omitted, and the following procedure may be used.

To a 10 ml. aliquot of the diluted digest, 2 ml. of sodium potassium tartrate solution is added, followed by the dropwise addition of concentrated ammonia until alkaline to acid metal cresol purple. Two drops of methyl red are added, a small piece of litmus paper is then introduced, and dilute ammonia (1 to 5) is added until the solution is alkaline to methyl red but acid to litmus (pH 5.0 to 6.0). Fifty ml. of the buffer solution is then added and the whole quantitatively transferred to a 250 ml. separatory funnel. Distilled water is added to bring the volume to about 75 ml. This solution will have a pH of 5.5 ± 0.2 , as measured by glass electrode.

The solution is now subjected to an "extractive titration." The standard dithizone solution is placed into a burette, previously washed with a little of the dithizone solution. Twenty-five ml. of the dithizone solution is added to the material in the separatory funnel, thoroughly shaken, and then allowed to stand. If the chloroform layer does not assume a bright red color, but remains green or has a purple color, there is no zinc or only a trace present in the sample. Should the chloroform layer, however, assume a bright red color, indicating zinc, then successive 25 ml. portions of dithizone solution are added to the material in the separatory funnel. After each addition, the separatory funnel is thoroughly shaken and then allowed to stand. Each 25 ml. portion of chloroform extract which assumes the bright red color of the zinc-dithizone complex is carefully removed and saved for confirmatory tests. This process of extraction and removal of the chloroform layer is continued until a chloroform layer is obtained that remains green or assumes an "off color" (purple). This titration furnishes an approximation of the quantity of dithizone solution required to extract all of the zinc.

The "extractive" titration of a second 10 ml. aliquot is conducted in the same way, except that 25 ml. less than the total volume of dithizone required in the first titration is added to the solution in the separatory funnel in one step. After thoroughly shaking, it is allowed to stand, and then the red chloroform layer is completely removed. The titration is then completed by successive 5 ml. portions of dithizone. The end point is reached when the chloroform layer does not assume a red color but remains green. It is not necessary to titrate with portions of dithizone less than 5 ml. because the maximum error involved would be only about ± 10 gamma of zinc. If greater accuracy is required, the titration near the end may be conducted by using one ml. portions of the standard dithizone. From the total volume of standard dithizone used in the titration of the second aliquot, the quantity of zinc present in the sample used may be calculated.

The zinc content may also be determined by combining all of the dithizone extracts, removing all free dithizone by washing with chloroform, then making

up to a definite volume and comparing the color with a standard zinc dithizonate solution by means of a visual colorimeter or a photo-electric colorimeter.

The presence of zinc may be confirmed as follows. The combined red chloroform dithizone layers are washed with 10 ml. of the buffer solution. The chloroform layer, containing the zinc is then shaken with 10 ml. of dilute hydrochloric acid (1 to 20), then drawn off and discarded. The acid aqueous layer is washed with three successive 10 ml. portions of pure chloroform, and then filtered. The filtrate is evaporated to about 5 ml., made alkaline with ammonia and then just acid with acetic acid. The resulting solution is divided into two portions. Into one, hydrogen sulfide is passed, a pure white precipitate indi-

TABLE 1
NORMAL ZINC CONTENT OF HUMAN LIVER AND KIDNEY

NORMAL HUMAN TISSUE, 25 GM.	MG. OF ZINC FOUND BY DIRECT DITHIZONE EXTRACTION	MG. OF ZINC FOUND BY DITHIZONE EXTRACTION AFTER PRELIMINARY H ₂ S SEPARATION
Liver.....	1.2	1.1
Liver.....	1.5	1.4
Liver.....	1.2	1.2
Kidney.....	1.4	1.2
Kidney.....	1.2	1.1
Kidney.....	1.6	1.3

TABLE 2

QUANTITATIVE RECOVERY OF KNOWN AMOUNTS OF ZINC ADDED TO 33 GM. SPECIMENS OF LIVER TISSUE HAVING A NORMAL ZINC CONTENT OF 1.6 MG.

NORMAL ZINC CON- TENT IN MG.	MG. ZINC ADDED	TOTAL ZINC CONTENT IN MG.	MG. OF ZINC FOUND BY DIRECT DITHIZONE EXTRACTION	MG. OF ZINC FOUND BY DITHIZONE EXTRACTION AFTER PRELIMINARY H ₂ S SEPARATION
1.6	2.0	3.6	3.9	4.2
1.6	1.0	2.6	2.6	2.5
1.6	3.0	4.6	4.5	4.5
1.6	4.0	5.6	5.8	5.7
1.6	2.0	3.6	3.0	3.4
1.6	1.0	2.6	2.7	2.9

cating zinc sulfide. To the second portion, potassium ferrocyanide solution is added, a white precipitate indicating zinc ferrocyanide. Finally a portion of the white sulfide may be subjected to spectrographic analysis, when the typical zinc lines will be evident.

In Tables 1 and 2 are charted some of the quantitative results obtained by the method described.

The experimental results listed in Tables 1 and 2 reveal the following facts:

1. If, as is usually the case, cadmium is not present in the tissues, the preliminary hydrogen sulfide separation may be omitted, thus simplifying the procedure.

2. The method is reasonably accurate for the quantitative determination of zinc in biologic material.

3. The normal zinc content of human liver and kidney tissue is surprisingly high, 5.4 mg. in 100 gm. of wet tissue. Cholak² reported finding from 0.5 to 1.5 mg. zinc in 100 gm. of normal tissue. His low values may be explained by losses due to volatilization, since dry ashing was employed.

SUMMARY

A simple method for detecting zinc in human tissues is described. The method may be used for the separation of zinc from all other common cations.

The dithizone "extractive titration" was applied to the quantitative estimation of zinc in tissues.

The method described is much simpler than the methods cited in the literature which are based on dithizone extraction.

The normal zinc content of human liver and kidney tissue is of the order of 5 mg. of zinc in 100 gm. of tissue.

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PREPARATION OF POTENT BLOOD GROUPING SERUMS*

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The use of purified blood group substance as antigen for the production of high titered antibody in human subjects was investigated in detail by Witebsky, Klendshoj and McNeil,¹¹ following widespread, if not correlated, observations that isoagglutinin titer tends to rise after accidental transfusion of incompatible blood. Aubert, Boorman and Dodd¹ reported a rise in isoagglutinin titer after transfusion with serum of an incompatible blood group. Also, Wiener, Soble and Polivka⁷ have prepared blood grouping serums by injections of partially purified saliva from secretors into appropriate human subjects.

During the two years since its inception, the Blood Grouping Laboratory of the Western Pennsylvania Hospital has produced all of its own grouping serum and has supplied such serum to other laboratories. This serum has been produced by the method of intravenous inoculation of selected donors (in this instance largely interns serving in the hospital) with 0.1 cc. Witebsky A and B Factors (Lilly) in 1 cc. sterile saline. After a ten day period, a trial bleeding is made and, if the isoagglutinin titer is satisfactory, from 150 to 250 cc. whole blood is bled from the donor. We use no serum with a titer of less than 4 plus in a dilution of 1:640; group B serum must be prepared against A₂ cells. During the two year period, 18 donors have been selected. It is our practice, at present, to inoculate a given individual only once. To date, we have seen no evidence of a reaction of any kind from the inoculation.

The variation in susceptibility to immunization from one individual to another is interesting. Of the 18 donors, only two were not susceptible to immunization with a single inoculation. These two individuals were both secretors. One individual among the 18 did not reach the requisite titer until two months after inoculation. Another donor reached a 4 plus isoagglutinin titer of 1:1280 in seven days. The initial titers of these donors before inoculation varied between zero, or no agglutination of appropriate cells by the undiluted serum, to a 4 plus reaction in a dilution of one part of serum in eighty parts saline. Most of the titers were found to range in dilution between 1:10 and 1:20. Titers of a potency high enough for use persisted in some instances for as long as six months after inoculation. Of course, unusually high titers of unknown origin exist normally in some individuals. One group O donor whom we had occasion to test had a 4 plus anti-A₂ titer of 1:640 dilution, with no previous sensitization, as far as could be determined.

Titer is determined by preparing from undiluted serum serial dilutions of 1:3, 1:5, 1:10, 1:20, and thereafter doubling the dilution up to 1:1280. Two drops of each dilution are incubated for thirty minutes at room temperature with two drops of the appropriate cell suspension. Then the tests are centrifuged lightly and read at once macroscopically.

* Received for publication, September 6, 1946.

After a suitable donor is bled, the agglutination rate of his serum is determined. Dilutions of 1:1, 1:3, 1:5, 1:10, 1:20 and 1:40 are made with saline, and an appropriate cell suspension is prepared. Slides are labeled, one for each dilution. One drop of the diluted serum is placed on the slide and to it is added one drop of the cell suspension. The slide is rotated and, with the aid of a stop watch, the interval determined between the time when the cells and serum were mixed and the first macroscopically visible agglutination. Again the time required to produce a 4 plus agglutination is observed. These two speeds of agglutination are plotted against the dilutions. It is interesting to note that there is usually an area of zoning; generally the 1:3 or the 1:5 dilution is the one which is most effective, or at which agglutination proceeds most rapidly. This dilution is chosen as the final one for use. The accompanying figures show representative curves obtained. Figure 1 shows an unusually rapid reacting antiserum. Figure 2 shows another within the normal range

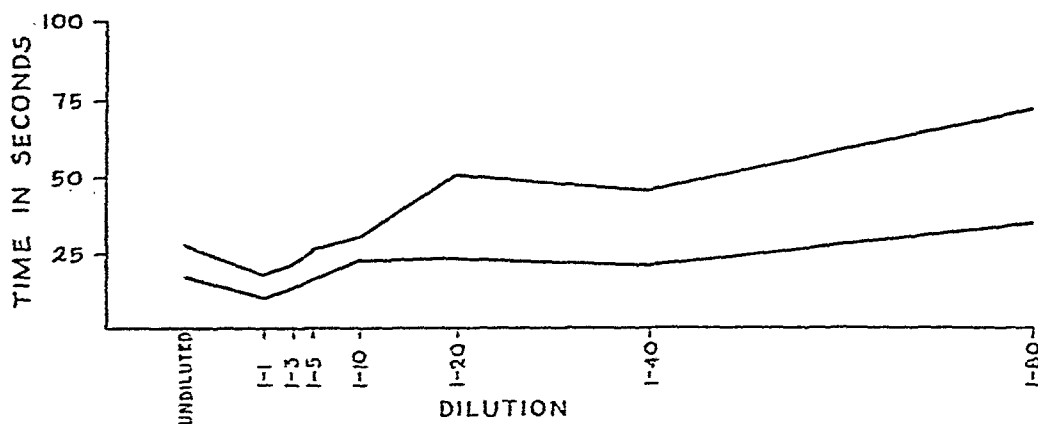


FIG. 1. UNUSUALLY RAPID REACTING HUMAN BLOOD GROUPING SERUM

Each of these serums gave a 4 plus reaction in a dilution of 1:1280 by the tube method.

The production of high antibody titer by the injection of blood group specific substance is of both theoretical and practical interest. Belkin and Wiener² used carefully prepared red cell stroma for intramuscular injections without untoward effects. Witebsky and Klendshoj^{8, 9, 10} considered the blood group specific substances to be carbohydrate in nature, *i.e.*, haptens in Landsteiner's sense.⁵ Theoretically, then, group specific substance should be capable of neutralizing antibody, but not of producing it in the experimental animal. That these substances will neutralize isoagglutinins, as demonstrated in relation to transfusions by Klendshoj and associates,⁴ and will produce antibodies on inoculation into the animal organism is obvious. Some investigators have been successful in avoiding difficulties with a series of injections of blood group specific substance into a single donor. Brand and Saidel³ found 6.1 per cent total nitrogen, 56 per cent reducing sugar and 30 per cent glucosamine in purified blood group "A" substance.

Perhaps the most interesting theory which attempts to account for an unex-

plained, yet apparently complete, antigenicity of carbohydrate material was advanced by Sevag.⁶ He suggested that a given carbohydrate, on injection into the experimental animal, may combine *in vivo* with a protein, thereby forming a complete antigen. It is possible that a reaction of this nature occurs upon the injection of group specific substance. Assuming for the sake of theoretical considerations that Sevag's theory of antibody formation is correct and that the action of antigen is that of a biocatalyst, it is possible that the requirement for a complete antigen is a holoenzyme and that, if either apoenzyme or coenzyme is lacking, either *in vitro* or *in vivo*, antibody formation is not stimulated. Thus, there would be conditions under which neither protein nor carbohydrate material would prove antigenic in relation to a specific substrate, whereas a combination of the two materials would provide the complete antigen.

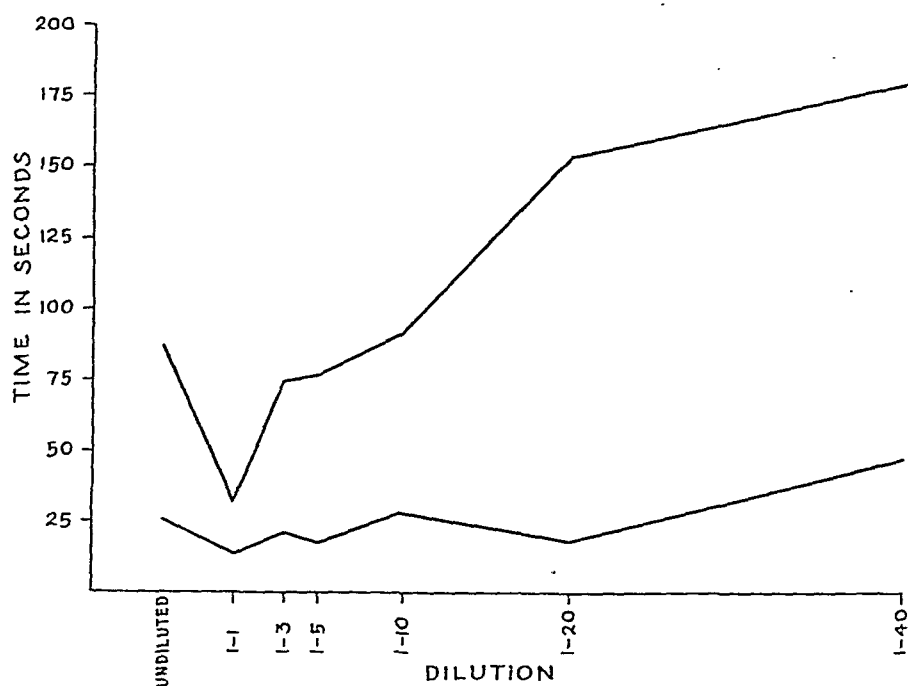


FIG. 2. REPRESENTATIVE TITRATION CURVE FOR HUMAN BLOOD GROUPING SERUM

SUMMARY

The methods of producing potent typing serum by intravenous inoculation of suitable donors with A and B factors have been reviewed. Titration methods and the determination of agglutination speeds are discussed and a theory selected to account for the antigenicity of the A and B substances.

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A COMPARISON OF RED BLOOD CELL COUNTING TECHNICS*

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There exist at the present time at least three methods of calculating erythrocyte counts. The Neubauer counting chamber method is the most commonly used and the most widely known. In 1943, Phillips *et al.*² published their technic of estimating the red blood cell count from the number of grams of hemoglobin as determined by their copper sulfate specific gravity method and Blum¹ introduced the photo-electric method of calculating the erythrocyte count.

In our work, Gowers' solution was used as the fluid in the counting chamber method and also as the diluent for the photo-electric colorimeter technic. Ch'U and Forkner³ demonstrated the superiority of this fluid in obtaining a more even distribution of the cells in suspension. Specimens of oxalated blood were collected from healthy and sick individuals. The number of erythrocytes was

TABLE 1
ERYTHROCYTE COUNTS, IN MILLIONS, OBTAINED BY THREE TECHNICS

NUMBER OF SPECIMEN	GRAVITY METHOD	CHAMBER METHOD	PHOTO-ELECTRIC METHOD
1	5.037	5.100	4.805
2	4.940	4.800	4.940
3	3.100	3.100	3.100
4	6.200	5.600	5.700
5	3.600	3.200	3.280
6	3.006	3.120	3.190
7	3.770	3.990	3.500
8	3.835	4.230	3.500
9	4.600	3.600	4.300
10	2.820	3.100	2.800

determined by the counting and gravimetric technics and estimated by the turbidimetric method.

Table 1 lists a series of cell counts, giving figures obtained by the three different methods.

Wintrobe⁴ states, "In spite of the fact that statistical analyses indicate that the error in counting red corpuscles may be as high as 16 per cent, even in the hands of trained technicians, it will be found that, with practice, counts agreeing within 200,000 per cu. mm. can be made." Using this limit of error as a criterion, our findings indicate a satisfactory correlation between the technics.

The simplicity of the turbidimetric technic not only enhances the reduplicability of the results, but reduces considerably the time and labor required. The gravimetric method is quite as simple, although not as rapid, and has the disadvantage of requiring a large quantity of oxalated venous blood and centrif-

* Received for publication, September 9, 1946.

ugation. The introduction of inaccuracies due to pipetting, dilution, shaking and distribution over the ruled area, as well as the time and labor involved in the performance of the chamber technic, render it the least desirable of the three methods.

SUMMARY

In a small series of erythrocyte counts, a close correlation of results was obtained by using the following three methods on each of the specimens of blood: copper sulfate specific gravity method of Phillips; photo-electric method of Blum; and the hemacytometer method, using the Neubauer ruling.

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A SIMPLIFIED METHOD FOR EMBEDDING CELLULAR CONTENTS OF BODY FLUIDS IN PARAFFIN*

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The recent publication of Hildebrand and Cheatele¹ emphasizes again the value of paraffin sections in the study of the cellular content of body fluids. There is, however, a much simpler method than the one they describe. The technic is not original with me, and although I do not know its source, I am reporting it because, despite its comparative simplicity, it gives excellent results and deserves much wider use. The essential feature is the use of the centrifuge, not only to concentrate the original fluid, but also to facilitate the changing of the various necessary reagents. The method is as follows:

1. Concentrate the fluid in the centrifuge tube, preferably in a conical one of 15 cc. Centrifuge, decant, add more fluid and centrifuge again until the sediment totals 0.3 to 0.5 cc.

2. Decant, add preferred fixative and mix with a glass stirring rod of small enough caliber to reach the bottom of the tube.

3. By centrifuging, decanting, adding the next fluid and mixing as above, pass through the various concentrations of alcohol and clearing agent to paraffin. It is usually sufficient to allow only about five minutes for each step.

4. After adding the first paraffin (no more than 5 cc. is needed), place tube with metal shield and reducing and trunnion rings in the oven. One change of paraffin is ample, and sufficient heat is retained by the metal shield and rings to prevent solidification of the paraffin during spinning. Finally, harden the paraffin in water. Prepare a pan of warm water slightly deeper than the length of the centrifuge tube, the temperature of which is somewhat above the melting point of the paraffin used. Place the tube in the water, and as soon as the paraffin begins to melt, remove the tube from the bath, invert and allow the entire mass of paraffin to slide from the tube into the hand. If this is done carefully, only the outermost portion of the paraffin will have melted. Cut off the end containing the sediment. Mount a small square of paraffin on a cutting block and dig out a hole just large enough to accommodate the sediment. Blend the paraffin of the block with that of the sediment by means of a warmed teasing needle. Cut, mount and stain.

REFERENCE

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* Received for publication, August 27, 1946.

AN IMPROVED HOLDER FOR NONPROTEIN NITROGEN DIGESTION TUBES*

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The holder to be described is used for nonprotein nitrogen determinations, when the digestion of the blood filtrate is carried out in 200 by 25 mm. pyrex test tubes. It has the following advantages: it does not break the tubes, has no rubber which may burn, places all tubes at the same height above the flame and is neat in appearance.

The parts necessary for the holder can be purchased locally at small expense. The following parts are necessary:

- One laboratory stand with $\frac{1}{2}$ " rod
- One face plate for lathe, 8" in diameter (used in sanding)
- One collar, with set screw, to fit rod of stand
- Six U-shaped holders (clothes closet pole holders)
- Twelve stove bolts $\frac{3}{16}$ " by $\frac{1}{2}$ "
- Three or six coil extension springs, $\frac{3}{8}$ " in diameter. (One may use three 3" carburetor springs cut in half.)

CONSTRUCTION

Six holes each 27 mm. in diameter are cut through the face plate, so that they are equally spaced and the outside edge of each hole is 15 mm. from the edge of the plate. The center hole of the face plate is drilled so that it will fit the rod of the stand (Fig. 1).

The U-shaped holders are bent slightly to fit the tubes properly, on one-half the perimeter, allowing 1 mm. clearance for variation in tubes and expansion due to heat. The inner curve of the U holder, with open end toward the center, is lined up with the outer curve of the 27 mm. hole on the under side, and the position for the bolt holes marked on the face plate through the tab on either side of the U holder. This is repeated for all six holes and the twelve small holes are drilled, having the same size as the hole in the tabs. Each U holder is bolted on loosely. A digestion tube is inserted and a spring placed across the open end of the U holder with at least two coils and two inches of straightened spring beyond. The tension on the spring is increased by putting more coils beyond the U and adjusted until the required position for use is obtained. The U holder does not bend; it is the spring tension that holds the tube. All except two coils of the spring beyond the U are straightened out and the free ends are bent down and fastened around the bolts which are then firmly tightened. It may be necessary to cut a small notch to keep the spring in the proper position at the point where it crosses the U holder.

* Received for publication, November 18, 1946.

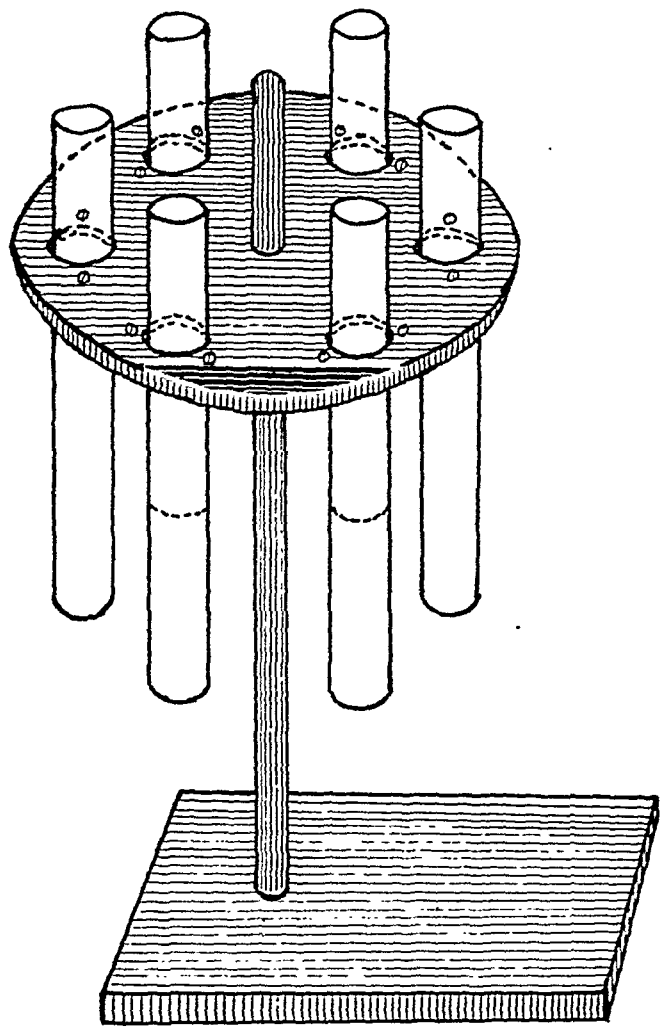


FIG. 1. TUBE HOLDER, AS IN USE

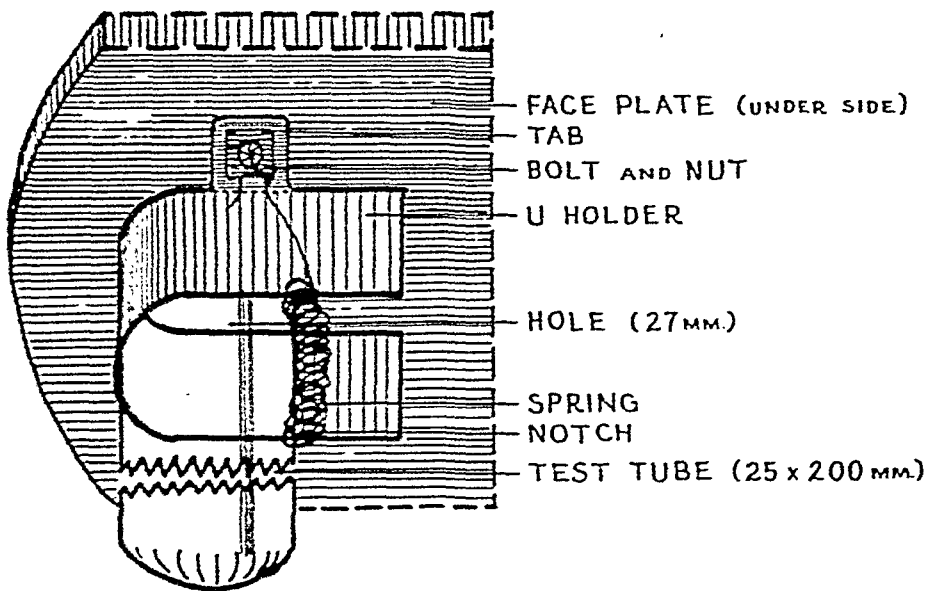


FIG. 2. SCHEME OF U HOLDING UNIT

A collar is placed on the rod and adjusted to keep the holder at the proper height when in use. To keep the metal parts from becoming too hot, it is preferable to place the greater portion of the tubes below the holder. When the digestion of one sample is completed, the face plate is rotated to bring the next tube into position over the flame.

SUMMARY

Construction has been described of a holder for nonprotein nitrogen digestion tubes which spares breakage and saves trouble and time.

THE DEVELOPMENT OF CLINICAL PATHOLOGY AS A SPECIALTY OF MEDICINE

ITS POSITION IN THE YEARS AHEAD AND THOUGHTS ON THE FUTURE TRAINING OF PATHOLOGISTS*

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FIRST STEPS IN ORGANIZATION

On May 22, 1922, the American Society of Clinical Pathologists was organized at St. Louis, Missouri. I am told that among those present at that meeting were Burdick, Hilkowitz, Corper, Hartman, Moore, Ivy and Wohl. I will not attempt a historic review of this Society, but I call attention to its organization as the first significant step in establishing clinical pathology as a distinct specialty of medicine. The second annual meeting was held here in San Francisco twenty-three years ago. An address, read at that time by Dr. Harry Gauss, was a scholarly historical presentation entitled "The Evolution of Clinical Pathology." Some of the Presidential Addresses since have been devoted to a description of the pathologist as a strange sort of person, so different from his medical colleagues. Several speakers have, in a sort of monody, classified him as they would a biologic specimen. Other addresses were jeremiads on the status of clinical pathology among associated branches of medical practice.

THE MORPHOLOGIC PATHOLOGIST

That the clinical pathologist received little recognition in those days is understandable. The teaching of Galen that "there is no disturbance of function without organic disease" was a concept still held by many. It is understandable that among pathologists and clinicians alike morphologic changes were *sine qua non* in the ultimate diagnosis of disease. The introduction of cellular pathology by Virchow in 1858 was a great step forward in an understanding of the processes of disease. It still is a highly important branch.

No one challenged, therefore, the status of the morphologic pathologist or the wisdom and accuracy of his diagnosis. His diagnosis has been admitted as the "last word," although I am not so certain it is always so. For a pathologist to suggest that he might offer the "first word" was entirely a different matter. Other physicians doffed their hats to the pathologist only in the deadhouse.

DEVELOPMENT OF ALLIED SCIENCES IN CLINICAL PATHOLOGY

In the years that followed Virchow, morphologic pathology reached its highest peaks. But slowly since that time, additions have been made to our knowledge

* Presidential address delivered at the Twenty-Fourth Annual Meeting of the American Society of Clinical Pathologists, San Francisco, California, June 28, 1946. Received for publication, January 8, 1947.

of bacteriology, physiology and biochemistry. Many bacteria and other living agents causing disease have either been actually isolated or demonstrated by one biologic process or another. The serologic tests for syphilis have offered more accurate and earlier methods of diagnosis than the morphologic manifestations. Indeed, although it is regrettable, the diagnosis of syphilis from its skin manifestations is becoming a lost art. How many times have extragenital chancres been missed by visual inspection? Modern therapy instituted early because of the greater diagnostic accuracy of such procedures as the Wassermann test, the darkfield illumination and the "punch biopsy," has prevented many of the later manifestations of syphilis which our predecessors were so accustomed to see. I have never seen in life such syphilitic lesions as Gross recorded fifty years ago in the wax figures now preserved in the Museum of Jefferson Medical College. The differential diagnosis of such luetic lesions must have been difficult and then accomplished late. Who would shun the serologic test today for the most expert opinion of yesteryear? Obviously I do not speak of an isolated case.

So it has become with other phases of medical diagnosis. I have, in recent years, watched many a leading clinician examine a patient and with a feeling of sadness have recalled the slow, painstaking and exact methods of McCrea, Fussell and Reisman. I think of Litten's diaphragmatic sign, the signs of Grocco, of Broadbent, of Kussmaul, and realize that the art of physical examination is on the wane. More exact methods yield more information. A properly exposed x-ray film reveals changes which the most expert clinician can not detect. The time has not come for the complete abolishment of physical diagnostic methods, and I believe, and teach, that the physician should by case history and physical signs arrive at a diagnosis to be confirmed or rejected by the more exact laboratory methods.

The recognition of bacterial infection is a task for the expert bacteriologist and serologist, possessing a full knowledge of the history and symptoms of the patient. One reads with admiration the detailed descriptions of the manifestations of such diseases as typhoid, typhus, or of Malta fever as they were written years ago. But these manifestations are late; and today we know of so many newly discovered fevers, such as the varieties of typhus, undulant fever and tularemia, that our diagnostic problems would be almost insurmountable were it not for the serologic tests available. So that while the expert clinician of today waits for the classical signs to develop, the laboratory often reveals the nature of the disease in a few hours. Again, I do not minimize the importance of a knowledge of the clinical aspects, but I emphasize the importance of the combined efforts of clinician, pathologist and radiologist.

Piece by piece we have seen the practice of medicine cut into specialties. A general practitioner today serves in part, at least, as a clearing house, turning this patient over to the surgeon, that one to the neurologist. Is it any wonder that he installs a basal metabolic machine and an electrocardiograph and hires a technician to perform such laboratory procedures as are profitable? He tried x-ray diagnosis, too, but in the main that was not profitable because of the cost of apparatus, and, as many have discovered, it was dangerous and required

considerable technical skill. But blood counts and basal metabolic determinations present no risk; who knows whether they are correctly done? A technician can be taught in a few weeks (so think some), and besides, she can help with patients and records. The greatest compliment such men can pay the clinical pathologist is to come to him when they themselves are ill. Such, however, is the behavior of human beings in an age of great economic competition.

The most noteworthy advances in clinical pathology have been made within the lifetime of most of us present here. The system of blood analysis had its beginning with the work of Folin and Dennis in 1912 and 1915. The improvements in technic published in 1920 by Folin and Wu were followed by the widespread use of this system in laboratories throughout the United States. The next fifteen years saw many notable advances, such as the flocculation tests for syphilis, quantitative agglutination tests for a variety of bacterial diseases, pneumococcus typing, renal and liver function tests, an understanding of the significance of blood sugar levels, of blood chlorides, calcium, phosphatase, and of the significance of the diameter of red blood cells in the classification of anemias. The increased demands upon the laboratory incident to these discoveries and new technics require personnel prepared by adequate instruction in the basic sciences, trained in the performance of intricate analytic methods, and directed by one familiar with the current literature and with the variations and complications caused by disease. Thus, clinical pathology has emerged from the ward laboratory and from the physician's office, to a position of great importance in the diagnosis and *control* of disease.

DEMAND FOR PATHOLOGISTS INCREASES

The demand for clinical pathologists has increased. Laboratories have grown in size and in personnel. The profession of medical technology was established wisely under the combined guidance of this Society and the American Medical Association. The Second World War established the laboratory and its entire staff as *indispensable* to any sort of medical practice. The military demand for technical help and professional supervision was greater than could be met by organized medicine. Pathologists and technicians were created in short training courses, unfortunately without due regard for personal fitness, adequacy of basic training and the length of time necessary for laboratory training. But there was an emergency to meet. It was admitted that a well staffed laboratory was essential even to a military hospital, and I think it will be further admitted that greater accuracy and efficiency resulted in a laboratory directed by one who had special training in this field. No further proof is required of the position of clinical pathology today.

Our economic problems are no different from those of medical men in general, or those of any other specialty. It was not until the latter part of the last century that any sort of limitations were placed upon those who could legally practice medicine. There is still no legal restriction to the practice of specialties among those licensed by the States. Despite opposition, various "pathies" have obtained a legal right to engage in questionable forms of practice. The treat-

ment of the sick can be a profitable enterprise and it seems as if the amount of profit is often in inverse proportion to the ethical standards and benignity of the practitioner. The attack upon these evils and upon *those that exist in organized medicine* must be largely one of education and demonstration. Legal attacks are necessary, but help from legal sources is weakened by the democratic principles of this nation, wherein an individual, regardless of his ability to do so, is allowed to choose his physician as he does his religion. It is further weakened by the reluctance of legislators to restrict or destroy established business. These benefits of democracy result from broad principles. Who among us would risk the abandonment of a single feature, except by making it unpopular through education?

CLINICAL PATHOLOGY RECEIVES OFFICIAL RECOGNITION

Clinical pathology has through the efforts of this Society traveled far. In 1930, a committee of the American Medical Association, investigating laboratory fees, wrote in its Journal: "pathologists or laboratory directors are different from surgeons and internists;" but a few years later, the Board of Trustees passed a resolution testifying that the practice of clinical pathology was the practice of medicine. A certifying board has been established clearly defining the qualifications requisite for establishing competency in pathology.

THE YEARS AHEAD

The years ahead promise greater expansion of the laboratory as a diagnostic and educational center. Conversant as he is with the language of all the other specialties, the clinical pathologist acquires a unique position. His position is most suited for direction of the intellectual activities of the group or hospital. Since his income is derived entirely from referred work, he can well serve as a public relations officer without the implication that he is "drumming up business" or seeking cheap advertisement. Since he devotes full time within the hospital and he contacts patients, both in ward and private room alike, he is most suited to serve as adviser to the administrative staff. He is in the most favorable position to offer service to the public, to the hospital staff, to his colleagues, and to the science of medicine. The clinical pathologist can make himself equal to such tasks.

THE PATHOLOGIST AS A CONSULTANT

The pathologist must consider his worth as a consultant and a correlator of clinical facts and laboratory findings. Often laboratory results can be correctly evaluated only with a full knowledge of the clinical signs and symptoms in a particular case. Such knowledge often suggests additional laboratory studies, which may not have occurred to the clinician, but which are important in the establishment of a diagnosis. Physical diagnostic methods and case study are not denied the pathologist and the use of these methods further establishes his position as a physician and earns for him greater esteem among clinicians and laymen alike. The direction of laboratory activities could fall into the hands of

a graduate in chemistry or even a well trained technician. Such persons soon begin to express opinions as to the normality or abnormality of a report. Most clinicians may draw conclusions from such reports about as successfully as they read x-ray plates. Such a practice would not be adopted by a wise clinician, but if some hospital administrators control the situation they are not beyond sacrificing scientific accuracy for administrative economy.

PREPARATION FOR THE YEARS AHEAD

The position of the clinical pathologist in the years ahead depends much upon his efforts in the five activities enumerated below.

1. *Organization.* The power of organized effort has been only too well demonstrated in labor circles in recent months. The cruel tools of labor organizations have no place in professional groups, but dignified pressure can be brought to bear upon institutions by a well organized national society. It has been proposed to form a new society for this very purpose. Whether interests of clinical pathologists can be best served by the American Society of Clinical Pathologists at less cost and without duplication of effort, or by a new group organized for the express purpose of fighting the economic battles of pathologists must be decided after careful consideration. Which ever course is adopted must have the support of every practicing pathologist.

Local societies must be formed in various communities, depending upon the number of pathologists concerned. Many such local organizations have been formed and are functioning. The local societies must maintain contact with the parent society by correspondence throughout the year and by delegations to annual meetings. Local societies should meet monthly during the year, if possible, for the discussion of scientific and economic problems. This is the method which has evolved so successfully in the American Medical Association. The thoroughness of organization will be a measure of the success to be anticipated. The time is near at hand when the National Society should support a full-time or part-time Executive Secretary. It is no longer fair to ask any of our members to make the sacrifice necessary to serve in this capacity, and the future will make demands on its executive officers even greater than ever before.

2. *Graduate Instruction.* Both the National Society and the local organizations must, by more frequent seminars, lectures, or bulletins keep every one of its members informed on the newer developments in technic. In the local groups, personal instruction by those of greater experience will improve the quality of practice of those of lesser experience. A gracious consultation service in the diagnosis of blood films, tissue sections and bacterial cultures will aid the younger man materially and will reflect credit on the members of our specialty as a whole. The offer by the larger laboratories to check analyses of the smaller institutions, or to supply standardized solutions, also will improve the quality of work done generally. These activities are not part of an idle dream for I have seen them put into effect in the Section on Clinical Pathology of the Philadelphia County Medical Society. Doubtless, other local societies are helping their members in a similar manner. The monthly publication in the Journal of well chosen con-

ference cases emphasizing the clinical and pathologic aspects might stimulate some men to correlate their findings in a similar manner.

The maintenance of a high standard of practice and the problems of the pathologist in relation to his hospital are primarily local problems but a powerful central organization must be prepared to render assistance when needed.

3. *Periodic Examination.* Some form of periodic examination of laboratories should ultimately be instituted by either the local groups or the National Society. This will serve to stimulate the maintenance of high standards of both the pathologist and the hospital administrative board. A series of unknown substances should be submitted from time to time and those laboratories failing in a proper evaluation of the unknown material should be so notified. I believe such periodic examinations are held for laboratories licensed in New York State. I do not know of any action that could be taken against institutions failing, but a published list of accredited laboratories might have a remarkable and powerful effect upon institutions not listed therein.

4. *Publication of Standard Methods and Values.* Another attempt to standardize technics and normal values should be made. There is great confusion today resulting from the multiplicity of methods and the varying results obtained therefrom. I think particularly of determination of values of phosphatase and hemoglobin. The results obtained in one institution may not be comparable with those of another in the same town or city.

5. *Lay Education.* This field of activity has been greatly neglected as a Society enterprise. Carefully selected articles might be published in *Hygeia* or in one of the many monthly periodicals. The public is quite uninformed on the activities of pathologists and it is so eager to learn. Such articles would be very widely read if properly written. Both the National Society and the local organizations can accomplish much through addresses to lay organizations. Women's clubs, service clubs, church and fraternal organizations eagerly accept offers of good speakers on medical subjects. Contacts can be made through speakers' bureaus maintained by most county medical societies. Once a good address is delivered requests soon follow for more. This is largely a matter of individual effort but such effort can be stimulated by the National Society.

TRAINING OF PATHOLOGISTS

In order to keep pace with the program outlined above, the pathologist of the future must be better trained. Something is definitely wrong with the basic and the medical training of young men today. I encounter so many interns and residents who have not the slightest idea of the use and care of a microscope, not the slightest conception of the optics of a darkfield illuminator. Many blink at a request to work out a calibration curve for a photo-electric colorimeter. Some candidates for certification by The American Board of Pathology could not list the tests employed to study liver function, although they are familiar with most of the individual tests. They have never learned to think of these tests in a group. Few have any conception of the meaning of a statistical analysis. Just where the difficulty lies I do not know. College and medical school entrance

examinations seem severe enough to permit selection of the most capable. Of one thing I am certain: much time is wasted in the present medical school curriculum. Many of the lectures could be abolished and practical laboratory or conference courses substituted. I believe there is little of real teaching value in the surgical amphitheater or in the "surgical assistantships" for medical students. Clinical clerkships, insisted on by the Council on Medical Education, are to be hailed as one of the wisest advances in medical student instruction.

Clinical and pathologic conferences can be as instructive for advanced medical students as for graduates. It is in the conference that the medical student may learn of the specialty of clinical pathology. Is it any wonder that students who have never had a classroom contact with a clinical pathologist fail to register a desire to enter this specialty? Here is an opportunity for some missionary work. A judiciously written pamphlet for medical students would stir up considerable interest.

The laboratory service for hospital interns is likely to induce a dislike for clinical pathology rather than a desire to make a specialty of it. In some institutions the intern serves as a substitute technician, doing much of the routine work with little actual opportunity for clinical correlation of laboratory data. I am opposed to interns serving in any routine capacity except for a time just long enough to demonstrate the principle of each laboratory procedure. In some phases this might require the entire period of his service, as for example, the gross description of surgical specimens, or the performance of autopsies. The latter procedures always should be under direct supervision. I plan to have the intern devote the major portion of his time to observation and directed study.

I strongly recommend an additional year of university study in the subjects of chemistry, physics and biology for those who contemplate specializing in clinical pathology. One of three resident years might be so spent with great profit.

Finally, I believe due consideration should be given to improving the broad intellectual talents of the resident, his literary efforts, and his ability to meet his colleagues, his superiors and the public with confidence and dignity. May we lead the way by good example.

LEUKEMIA (SUMMARY OF 100 CASES) AND LYMPHOSARCOMA COMPLICATED BY PREGNANCY.

CELLULAR CHANGES PRODUCED IN GUINEA PIGS BY EXTRACTS OF "LEUKEMIC" PLACENTA

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The purpose of this paper is to report: (1) an additional case of chronic myeloid leukemia complicated by pregnancy; (2) the completion of a case of myeloid leukemia previously reported;¹ (3) an additional case of lymphosarcoma complicated by pregnancy; and (4) the cellular changes found in the organs of guinea pigs injected with lipid extracts obtained from a normal placenta and from the placenta of a patient with chronic myeloid leukemia.

REPORT OF CASES

Case 1. Myeloid leukemia complicated by pregnancy

Clinical data. A 25 year old white housewife was referred to Jefferson Hospital by Dr. Oscar Wood on September 20, 1944, because of splenomegaly, fatigue and bleeding from the gums. The patient had noted a mass in the region of the spleen a year previously, but because it was not associated with symptoms she had not consulted a physician. The patient had two children, aged 2 and 4 years, respectively. The patient's father had died at the age of 54 of pernicious anemia. The patient was a slight, dark-haired, pale individual weighing about 120 pounds. There were small clots of blood at several points on the margins of the gums. There was slight generalized lymphadenopathy. The inferior hepatic edge could be felt 1 inch, and the splenic edge 2 inches, below their respective costal margins.

Laboratory findings were as follows: hemoglobin, 60 per cent (Klett); erythrocytes, 3,100,000 per cu. mm.; platelets, 200,000; leukocytes, 190,000; differential count in per cent: polymorphonuclears, 55; metamyelocytes, 32; myelocytes, 7; basophils, 3; lymphocytes, 3; prothrombin time 35 per cent of normal; basal metabolic rate, plus 17 per cent; blood uric acid, 4.8 mg. per 100 cc. X-ray therapy was directed to the adrenal glands. From October 6, to October 14, 1944, she received a total of 400 r.

Course. Menstruation did not occur during the month of January 1945. In March 1945, a positive Friedman test was obtained. It was believed that the patient should have been aborted, but she refused. By March 1945, the white blood cell count had dropped to 20,000 but in May 1945, it had risen to 80,000. Fowler's solution was started then because of the danger of exposing the fetus to x-ray irradiation. The leukocytic level remained below 100,000 until September 1945, when it rose to 172,000 and was associated with 20 per cent myeloblasts in the differential count. During October 1945, the ninth month of pregnancy, the white blood cell count rose to about 400,000 despite the continuous use of Fowler's solution. During the first twenty-eight days of October the patient received fifteen blood transfusions (500 cc. each). On October 29, the patient was delivered by Dr. J. E. Lynch of a 6 pound, 6 ounce normal boy. A differential leukocyte count of the cord blood revealed the following percentages of cells: polymorphonuclears, 55; eosinophils, 5; lymphocytes, 38; monocytes, 2 along with 28 per cent normoblasts. Blood count of the baby revealed the following: hemoglobin, 105 per cent; erythrocytes, 4,500,000 per cu. mm.; leukocytes, 19,850

* Received for publication, January 4, 1947.

per cu. mm.; differential count in per cent: polymorphonuclears, 66; eosinophils, 3; basophils, 1; lymphocytes, 25; monocytes, 5; (normoblasts, 2). During the two weeks the baby remained in the hospital, five complete blood counts were made and the findings in all of



FIG. 1. LEUKEMIC INFILTRATIONS OF SKIN, CASE 1.

them were within the limits of normal. The placenta weighed 550 gm. and, on section, was histologically essentially normal except for some slight infiltration of the maternal lacunas with myelocytes. The uterus was packed with gauze, soaked in thromboplastin, fibrinogen and adrenalin and the cervix sewed because of profuse uterine bleeding. During the first seven hours following delivery, the patient received intravenously seven pints of blood. Despite the transfusions and the cessation of bleeding, the blood findings on the next morning were as follows: hemoglobin, 39 per cent; erythrocytes, 1,700,000 per cu. mm.; leuko-

cytes, 169,000 per cu. mm.; differential count in per cent: polymorphonuclears, 27; metamyelocytes, 17; myelocytes, 19; blasts, 26; lymphocytes, 5; monocytes, 6. The uterine pack was removed November 1, 1945 and no bleeding followed. The white blood cell count rose to 400,000 by November 12, when 5 millicuries of radiophosphorus was injected intravenously. The leukocyte count gradually decreased to 70,000 by December 5, 1945. The patient developed some uterine bleeding (platelets 130,000) and radium (2400 mg. hr.) was applied to the uterine cavity and several transfusions were administered. On December 22, 1945, the patient developed leukemids over the entire body and the breasts became stony hard (Fig. 1). The leukemids were purplish in color, were elevated and many of them each measured about 1 cm. in diameter.

The patient was readmitted to hospital January 14, 1946 complaining of bleeding gums, tender, hard breasts, "purple spots" in the skin, and partial blindness of the left eye. Physical examination revealed large retinal hemorrhages in both eyes, bilateral subconjunctival hemorrhages, generalized lymphadenopathy, leukemids in most of the skin of the body, and the spleen was palpable $4\frac{1}{2}$ inches below the left costal margin.

Laboratory findings were as follows: hemoglobin, 37 per cent; erythrocytes, 1,680,000 per cu. mm.; platelets, 170,000; leukocytes, 284,000 per cu. mm.; differential counts in per cent: polymorphonuclears, 26; metamyelocytes, 19; myelocytes, 21; blasts, 25; lymphocytes, 7; and monocytes, 2; urea clearance, 42 per cent of normal; blood proteins, albumin 4.2 gm. and globulin, 1.3 gm. per 100 cc.

Course. The daily peak of temperature ranged from 102 F. to 104 F. Transfusions were given three times a week. The white blood cell count rose to nearly 600,000 per cu. mm. before the patient expired on March 5, 1946. During the last year of her life, she had received fifty-two transfusions.

Post mortem. The autopsy findings were typical of myeloid leukemia. Hemorrhages were observed in the sclera and in the skin of the chest and abdomen. The left pleural cavity contained 2000 cc. of straw-colored fluid. All organs appeared infiltrated. The spleen weighed 950 gm. and the liver 2460 gm. The uterus was normal in shape and size. The lungs were congested and edematous. The histologic findings follow: myeloid cell infiltration of nearly all organs and tissues; extensive and marked infiltration of the skin; fibrosis and infiltration of the breast; considerable infiltration of ovaries and uterus; pulmonary congestion and edema.

CELLULAR CHANGES IN GUINEA PIGS FOLLOWING INJECTION OF EXTRACT OF "LEUKEMIC" PLACENTA

As stated above, the placenta of Case 1 was delivered October 29, 1945, weighed 550 gm. and, histologically, was essentially normal except for slight leukemic infiltration and for a few myelocytes in the maternal lacunas. It was ground up and mixed with 3 liters of methyl alcohol acidified by hydrochloric acid and allowed to stand overnight. A normal placenta (Mom), which weighed 850 gm., was obtained the following day and it, too, was extracted identically with acidified wood alcohol. The alcoholic extract of the normal placenta and that of the placenta of Case 1 were treated identically as follows. The alcoholic extract was filtered and then extracted with petroleum ether. The ether extract was extracted by an alcoholic solution of potassium hydroxide (300 gm. potassium hydroxide in 1000 cc. methanol). To this extract was added hydrochloric acid until acid to congo red paper. The solution was then extracted with ethyl ether. The ether was evaporated and 0.4 gm. of a dark brown oily highly odorous material was thereby ultimately obtained from the

placenta of Case 1 and 0.3 gm. of a similar appearing substance was obtained after the normal placenta was so treated.³⁸

One-tenth gm. of the extract of the leukemic placenta was injected subcutaneously into each of two guinea pigs²⁷ and the injections were repeated a week later. The extract of the normal placenta was likewise similarly injected. All four guinea pigs were sacrificed three weeks after the second injections were administered and all organs were studied histologically.¹⁰ The organs of both guinea pigs receiving the extract of the normal placenta were normal; but of the two animals receiving the extract of the leukemic placenta, one had slight myeloid infiltrations in the liver and kidneys, while the other had signifi-

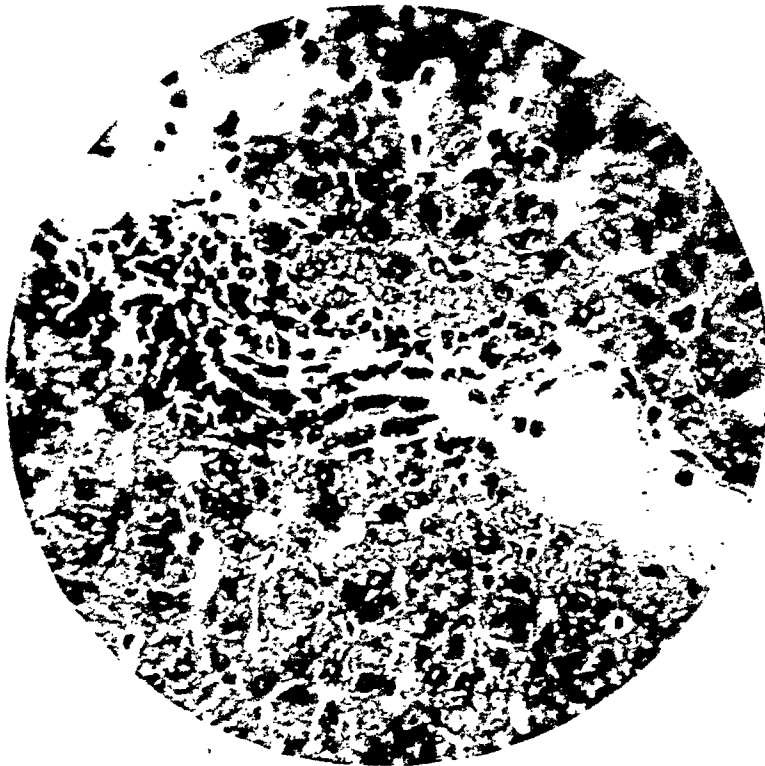


FIG. 2. MYELOID CELL INFILTRATION OF LIVER OF GUINEA PIG THAT RECEIVED INJECTIONS OF LIPID EXTRACTS OF THE PLACENTA OF PATIENT WITH CHRONIC MYELOID LEUKEMIA, CASE 1.

cant myeloid cell infiltrations in the liver (Fig. 2), the kidneys and spleen, and the marrow was hyperplastic. These findings indicate that although the placenta of Case 1 was histologically not markedly leukemic the potentialities of experimentally producing a "myeloid reaction"²⁸ were still present.

In October 1946, the male child born of Case 1, on October 29, 1945, was alive and well.

Case 2. Myeloid leukemia complicated by two pregnancies

Clinical data. This case was first reported¹ in 1944 after the leukemic patient delivered her first child, but is mentioned again to relate the fatal outcome of the patient and her two offspring. The patient, 22 years old, was first seen by Dr. H. M. Angelucci, in December

1942, at which time she had been pregnant for two months. In June 1943, when the white blood cell count was 126,000, the patient received x-ray therapy directed to the anterior and posterior aspects of the thighs and chest. In July, a 7 pound, 2 ounce hydrocephalic male infant was delivered. In July and August of 1943, the patient received x-ray therapy directed towards the spleen and femurs. The patient became pregnant the second time in January 1944. During the pregnancy the leukocytes rose to 400,000 per cu. mm. and the patient received additional x-ray therapy directed to the spleen and long bones. Dr. Angelucci delivered the second baby in September 1944. The postpartum course was complicated by much bleeding and by poor healing of the perineum. The patient was sterilized by ten x-ray treatments to the ovaries in December 1944. On March 28, 1945, the patient was admitted to Jefferson Hospital complaining of weakness, nausea and vomiting, loss of 50 pounds of weight during the preceding ninety days and nonproductive cough of thirty days' duration. On physical examination, the patient was pale, slightly jaundiced, emaciated and dark complexioned. Cold beads of perspiration were present on her forehead and she coughed intermittently and nonproductively. The temperature was 103 F., the pulse rate 120 and the respiratory rate 26. A soft blowing systolic murmur was heard over the mitral area. The spleen was hard and extremely large, the inferior tip extending into the pelvis. The liver was palpable 2 inches below the right costal margin. There were small hemorrhoids present. Laboratory findings were as follows: hemoglobin, 26 per cent; erythrocytes, 1,320,000 per cu. mm.; platelets, 38,000 per cu. mm.; leukocytes, 116,000 per cu. mm.; differential count in per cent: polymorphonuclears, 11; metamyelocytes, 20; myelocytes, 23; blasts, 16; basophils, 15; basophilic myelocytes, 12; and lymphocytes, 3; bilirubin, 4.3 mg. per 100 cc.

Course. In spite of transfusions, liver extract and penicillin for inflamed pharynx, the fever persisted, the blood findings changed little and the patient died May 26, 1945.

Post mortem. The anatomic findings were typical of leukemia. The spleen weighed 3940 gm. and had many infarcts and perisplenic adhesions. The liver weighed 4600 gm. The gallbladder was distended and contained several stones. There were also bilateral hydrothorax, atelectasis of the lungs and an infarct of the right upper lobe. The histologic findings were: myeloid cell infiltration of nearly all the organs; extramedullary myelopoietic foci in sinusoids of the liver; bronchopneumonia; and venous congestion in the lungs.

Offspring of Case 2

The first child, born July 21, 1943, was admitted to Jefferson Hospital, July 15, 1945, with the complaint of irregular continuous fever since birth. The fever seemed to be related to the room temperature according to caretakers, who also informed us that the parents had been first cousins. On physical examination, the boy had a large hydrocephalic head, but otherwise seemed fairly well developed. A bruit was heard over the left carotid artery.

Laboratory findings. Hemoglobin was 61 per cent; erythrocytes, 4,080,000 per cu. mm.; platelets, 160,000 per cu. mm.; leukocytes, 7,900 per cu. mm.; differential count in per cent: polymorphonuclears, 33; eosinophils, 1; lymphocytes, 60; and monocytes, 6. The sternal marrow findings were essentially normal. The cerebrospinal fluid was clear and under 75 mm. of water pressure. Tests revealed chlorides, 781 mg.; proteins, 120 mg.; Wassermann, negative. There were 6 leukocytes per cu. mm. X-ray studies of the long bones were normal. The patient's mental age was much below normal.

Course. There was a spiking fever that reached 102 F. daily. There was a definite correlation between the room temperature and the patient's body temperature. An attempt was made to obtain an arteriogram of the internal carotid tree. The patient died two days later, on September 5, 1945.

Post mortem. The anatomic diagnoses were hydrocephalus, pulmonary edema and congestion. The brain weighed 1300 gm. The convolutions were flattened and the sulci obliterated. The spleen weighed 60 gm. and the liver 440 gm. The histologic diagnoses were:

immature development of the cortex of the brain and congestion of the brain, lungs, kidneys and spleen.

The second child, as noted above, was born in September 1944 in Women's Hospital. Five days after birth, the child's temperature rose to 107 F. and on the sixth day to 110 F. An autopsy was performed by Dr. B. Meine who felt that the child might have had toxoplasmosis.

Both of the offspring of the patient in Case 2 apparently had some disturbance of their thermal centers. Were these disturbances due to the x-irradiation received by the mother? The placentas were unfortunately not examined histologically.

Case 3. Lymphosarcoma complicated by pregnancy

Clinical data. A 34 year old colored housewife was referred to the Elizabeth Storck Kraemer Foundation, Jefferson Medical Hospital, April 22, 1942, as an out-patient because of an enlarged left cervical lymph node of two months' duration, which on biopsy was reported as typical of Hodgkin's disease. The patient also complained of generalized itching. Physical examination was essentially negative except for the enlarged left cervical node which measured about 3 cm. in diameter. All of the laboratory findings were negative except for x-ray evidence of enlarged mediastinal nodes.

Course. The patient received a total of 4000 r. of x-radiation directed to neck and chest during the months of May, August and December 1942; in April and August of 1943, she received an additional 3000 r. Dr. W. H. Kraemer examined the patient in August 1943 and found evidence that she was pregnant. A positive Friedman test was obtained in September. On November 24, during the fifth month of pregnancy, a hysterotomy was performed on the patient in the Philadelphia Lying-In Hospital. Neither the fetus nor placenta were studied histologically. On January 14, 1944, the patient entered Pennsylvania Hospital in a moribund state. Physical examination revealed an extremely emaciated dyspneic 36 year old Negress with a blood pressure of 70/50. The spleen extended 2 inches and the liver 4 inches below the costal margin. Laboratory findings were: hemoglobin, 71 per cent; erythrocytes, 3,200,000 per cu. mm.; leukocytes, 13,000 per cu. mm.; differential, normal. The patient died on the third day after admission.

Post mortem. The anatomic findings were: extensive lymphosarcoma of mediastinum which involved the periaortic lymph nodes and extended into the right lower lobe of the lung, and by following the portal vein, into the liver and pancreas. The spleen was enlarged and the bone marrow hypoplastic. Histologically, the tumor was a "collection of atypical whorls of spindle-shaped cells with bizarre-shaped and hyperchromatic nuclei which were associated with much reticulum."

The case began as Hodgkin's disease but ended as lymphosarcoma. This change or shift from Hodgkin's disease to lymphosarcoma, or vice versa, has been fully discussed elsewhere.¹⁵

DISCUSSION

Many hematologic dyscrasias have been complicated by pregnancy,⁴⁰ polycythemia,⁸ aplastic anemia,^{2, 23} purpura^{11, 33} and sickle cell anemia.³⁵ One hundred cases of leukemia complicated by pregnancy^{12, 13, 15, 25, 41} have been reported (Table 1). By slightly modifying the interpretations and data of some of the authors one may reach some rather practical, although rough, conclusions. There were 87 cases of myeloid leukemia (24 acute and 63 chronic) and 13 cases of lymphoid leukemia (10 acute and 3 chronic). Of the 34 acute cases, all died either before delivery or within less than three months after delivery; while of the 66 chronic cases, 43 survived more than one year after delivery. The chronic types of leukemia complicated by pregnancy are more

TABLE 1

A LIST OF AUTHORS REPORTING CASES OF LEUKEMIA COMPLICATED BY PREGNANCY, AS LISTED BY FORKNER,^{12, 13} GRIER,¹⁵ MCGOLDRICK²⁵ AND ERF.^{23, 41} IN REPORTS WHERE THERE ARE MORE THAN ONE AUTHOR, ONLY THE NAME OF THE FIRST IS LISTED FOR SAKE OF CONVENIENCE

FORKNER ^{12, 13} (1938)	FORKNER (CONTINUED)	GRIER ¹⁵ (1939)	MCGOLDRICK ²⁵ (1943)	ERF ^{23, 41} (1946)
Sanger	Lindbrones	Frank	Schenk	Kirstein ⁴¹
Jaggard	Joachim	Debiasi	Nanjoks	Bjore
Laubenberg	Kaplan	Rydzewska	Baldridge	Williams ^{41p}
Hilbert	Allan	Zanela	Haining	Forsyth
Ashanazy	Lammers	1937 (2nd)	Langer	1928
Lazarus	Neumann	1938	Bentivoglio	1936
Bostetter	Geller	Grier	Isaacs	1939
Savarre	Neumann	Hauch	Kandel	Tschopp ⁴¹
Savarre	Ridder		Erf	1941
Kleineberger	Mann		Erf	1941 (2nd)*
Melinkow	Held		Zambonini	1941†
Gasser	Weber		Herrenberger	1942
Thaler	Hofstein		Harrison	1942
Peterson	Saidl		Murphy	1943
Ward	Recek		McGoldrick	1943
Marmal	Saidl			1944
Kosmak	Kaplan			1944
Renon	Heim			1944
Kosmak	Neumann			1944 (2nd)
Wallgren	Russell			1945
Meurer	Hussy			1945 (2nd)
Hausman	Garrasi			1946
Fleishmann	Hussy			1946
Ohlsson	Mehta			1946
Bower	Brandstrup			1946
Thamer	Traina			1946
Bower	Forkner			1946
Chiari				

CASES IN WHICH DATA WERE INCOMPLETE, DOUBTFUL, OR NOT OBTAINABLE

FORKNER (1938)	GRIER (1939)	MCGOLDRICK (1943)		EHRF (1946)
		Galabin	1901	Tanton
Paterson	Sutcliffe		1914	
Ingle	Jimenez		1921	
Cameron	Nagy		1924	
Greene	Bruggeman		1928	
Stillman	Labhardt		1932	
Schroeder	Pontoni		1937	
Merttans	Donati		1937	
Herman				
Brandt				
Schreiner				
Sargent				

* The two cases listed in reference⁴¹ were 21 and 38 years of age, respectively. The diagnosis of chronic myeloid leukemia was made in both during the latter months of pregnancy. Both patients delivered normal children. The former was given orally 41 millicuries of radio-phosphorus and the latter 60 millicuries before death. The postmortem findings of the former were typical of myeloid leukemia (the bone marrow had many megakaryocytes), while those of the latter were myeloblastic.

† A personal letter from Dr. G. C. Bates, Independence, Kansas, dated August 20, 1946, indicated that a normal healthy boy was delivered from his patient with chronic myeloid leukemia by cesarian section on January 10, 1942. The patient died June 6, 1942, but the child at 4½ years of age was in perfect health.

frequently of the myeloid type. (It has been stated that the ovaries are not as intensely infiltrated in myeloid leukemia as in lymphoid leukemia.) Excluding abortions and maternal deaths before delivery, less than 50 per cent of the babies will be normal in cases of acute leukemia, but nearly 75 per cent of the babies will be normal in cases of chronic leukemia (Table 2). The evidence indicates that the course of leukemia is not altered by pregnancy; that in acute leukemia, abortions are not indicated because of the acute course of the leukemic process; that in chronic leukemia, abortions are not indicated because of the high percentage of normal offspring. There has been no report of a leukemic newborn delivered of a leukemic mother, although there have been reports^{5, 17, 22, 30} of leukemic newborns delivered of hematologically normal mothers. The placentas are normal³⁰ in the latter. Pregnant leukemic mice deliver normal young.³ There is no consistent evidence that pregnancy increases the activity of leukemia, lymphosarcoma or Hodgkin's disease, or that, in these diseases, it hastens the death of the mother.

TABLE 2
ANALYSIS OF BIRTHS FROM MOTHERS WITH ACUTE AND CHRONIC TYPES OF LEUKEMIA

	ABORTIONS (BOTH INDUCED AND SPONTANEOUS) AND FETAL DEATH DUE TO MATERNAL DEATH	STILLBIRTHS	BABIES THAT LIVED LESS THAN 2 YEARS	NORMAL BABIES
Mothers with acute leukemia...	12	12		9
Mothers with chronic leukemia..	17	7	5	37
	29	19	5	46

The cause of leukemia is unknown. The theoretical mechanism that seems most logical at present is that proposed by F. R. Miller.^{28, 29} He implies that acute leukemia is due to a lack of a specific maturation factor. Cooke has a similar theory.⁴ Viruses and bacteria have frequently been implicated as causes, but as yet leukemia has never been transmitted in human beings.³⁷ It is possible, however, that intestinal bacteria could supply the maturation factor needed. Except for the lymphemic aspects of some malignant processes, as lymphosarcoma, leukemia is not cancerous. And although there is a rare familial instance,^{6, 12, 19, 26} leukemia is not inherited. Some of the other causes of leukemia that have been presented are abnormal hormones (adrenal hypo-activity increases lymphopoiesis^{7, 31}), cosmic rays, hypofunction of the spleen and emotional excesses.

Because the causes of leukemia are unknown, there are many types of treatment. Fowler's solution, which contains arsenic, is probably an enzyme inhibitor and has been used in cases of leukemia complicated by pregnancy because of the rather large margin of safety offered the fetus. There is much evidence that x-radiation directed toward the pelvis can be harmful to the fetus; possibly

the two offspring of Case 2 were injured by x-radiation. X-radiation directed towards areas other than the pelvis and abdomen, probably would not harm the fetus. In Case 1, before pregnancy occurred, x-radiation was directed toward the adrenal glands in an endeavor to reduce their function so that more lymphocytes might be released.³¹ So far, radiophosphorus⁴¹ has been used twice* in leukemia complicated by pregnancy. Because it emits beta rays it probably is less dangerous to the fetus than x-ray. It does not alter ovulation because a patient with polycythemia,⁹ who desired pregnancy greatly, became pregnant about two months after the intravenous administration of 5 millicuries of radiophosphorus (P^{32}). The administration of large amounts of yeast seems to have a place in the treatment of leukemia because yeast neutralizes, or aids in the conjugation of, the substances stimulating the white cells to abnormal growth. Urethane^{16, 32} and nitrogen mustards³⁴ have not been used extensively enough in leukemia to draw definite conclusions.

The histologic findings in placentas from mothers with leukemia and from mothers with leukemic newborn babies are rarely discussed. In instances of the former, the placentas examined have rarely been infiltrated with leukemic cells but some immature cells have been observed in the maternal lacunas.⁴¹ Apparently in instances of congenital leukemia, the placentas are normal.³⁹ It would seem, therefore, that the placenta is a distinct barrier to leukemia, and without regard to the side of the placenta on which the leukemia exists.

There are several reports of cases of Hodgkin's disease complicated by pregnancy^{14, 23, 24, 40} but few reports of lymphosarcoma.^{21, 35} The biopsy of the cervical node of the patient of Case 3, in April 1942, was typical of Hodgkin's disease, but at death, in January 1944, the lesions were typical of lymphosarcoma. Herbut, Miller and Erf¹⁸ have seen such changes frequently and believe that the changes are due to varying amounts of lymphokentric and myelokentric acids circulating in the body.³⁹

SUMMARY

An additional case of chronic myeloid leukemia complicated by pregnancy is reported. The patient had received 52 transfusions during the last year of her life. A photograph of leukemids and the autopsy findings are presented.

A second case of chronic myeloid leukemia, previously reported, is completed. The autopsy findings of the mother and two offspring are discussed. The parents had been first cousins. Both children died apparently of disturbances of the thermal center, possibly brought on by x-radiation of the mother during pregnancy.

A brief analysis of 100 cases of leukemia complicated by pregnancy is presented. There were 87 cases of myeloid leukemia (24 acute, 63 chronic) and 13 cases of lymphoid leukemia (10 acute, 3 chronic). Less than 50 per cent of babies of mothers with acute leukemia are normal, while 75 per cent of babies whose mothers have chronic leukemia are normal.

* See first footnote to Table 1.

An additional instance is reported of lymphosarcoma complicated by pregnancy. In this case, the findings at biopsy were those of Hodgkin's disease and at autopsy, those of lymphosarcoma.

Lipid extracts of a normal placenta and of one obtained from a patient with chronic myeloid leukemia were injected into two guinea pigs. A photograph is presented which shows myeloid cell infiltration of the liver of a guinea pig which had been injected with the extract of the "leukemic" placenta.

CONCLUSIONS

The course of leukemia is not altered by pregnancy. Fowler's solution, transfusions and brewer's yeast are still probably the best therapeutic agents in leukemia complicated by pregnancy. X-radiation directed even close to the abdomen is probably dangerous to the fetus.

The placenta from a patient with chronic myeloid leukemia showed slight infiltration with myeloid cells. A lipid extract of this placenta, injected into two guinea pigs, produced myeloid cell infiltrations in the organs, while injection of a similar extract of a normal placenta produced no abnormal cellular infiltrations in the organs of two control animals.

The placenta is a complete barrier to leukemia, whether the leukemia is in the mother or in the fetus.

Acknowledgments. I wish to thank Dr. Franklin R. Miller and Dr. Daniel L. Turner for valuable assistance.

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A NONINFUSION BLOOD AGAR BASE FOR NEISSERIAE, PNEUMOCOCCI AND STREPTOCOCCI*

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Blood agar is the most important culture medium in the clinical laboratory. It is especially valuable for the detection of pathogenic cocci, many of which have the ability to lyse or discolor blood, and for the detection of organisms possessing fastidious growth requirements. When heated in the basic medium, chocolate agar is obtained. This is employed extensively for the isolation and cultivation of *Hemophilus influenzae* and *Neisseriae gonorrhoeae*. While fresh meat infusion has been generally considered best suited as a base for these mediums, the inconvenience and time involved in its preparation make a more simply prepared medium desirable. The purposes of the studies described herein were first, to find a dehydrated base to supplant the fresh meat infusion base, and second, to develop a single blood agar medium which would possess the properties of both unheated and heated (chocolate) blood agars. Such a medium would lessen considerably the time and effort spent in the preparation of mediums, ensure use of the appropriate medium and improve detection of unsuspected pathogens.

In the development of such a medium, one must consider not only the ability to support growth of fastidious organisms, but also the maintenance of typical colony morphology and the production of hemolysis and of green coloration. Improvement of the reliability of surface inoculation, as compared with the cumbersome pour and streak-pour methods for the detection of alpha- and beta-hemolytic and gamma streptococci, is important. Although the value of the pour or streak-pour blood agar plate for the detection of some of the Lancefield serologic groups of streptococci has been established,^{1, 2, 13} the studies by Pike¹² showed no definite advantage for streak-pour plates over streaked plates for the detection of hemolytic streptococci when infusion agar containing human blood was employed.

I. DEVELOPMENT OF A DEHYDRATED BASE FOR BLOOD AGAR TO REPLACE FRESH MEAT INFUSION

Wright's¹⁴ base containing fresh beef infusion was used as a standard for comparison. Glucose was omitted from the medium because of its interference with hemolysis by beta streptococci. Of several peptones (Bacto-Peptone, Bacto-Neopeptone, Bacto-Proteose Peptone, Bacto-Proteose Peptone No. 3,

* The opinions or assertions contained herein are those of the author and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large. Received for publication, December 19, 1946.

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Bacto-Tryptose, Armour's Peptonum Siccum and a pancreatic digest of casein), Bacto-Neopeptone proved to be best suited for inclusion in this base, when test organisms including several strains of alpha- and beta-hemolytic and gamma streptococci, *Diplococcus pneumoniae*, *Brucella abortus*, *Neisseria gonorrhoeae*, *Hemophilus influenzae* and *Corynebacterium diphtheriae* were streaked on the completed blood agar medium (5 per cent human blood) and the amount and type of growth noted after eighteen to twenty-four hours' incubation at 37 C. The medium was markedly superior to any of the available dehydrated blood agar bases.

In the search for a suitable base prepared from dehydrated materials, it was found that a medium consisting of 2 per cent Bacto-Tryptose, 0.3 per cent beef extract, 0.5 per cent sodium chloride, 1.5 per cent agar and 0.03 per cent glucose

TABLE 1
EFFECT OF GLUCOSE AND ATMOSPHERE ON GROWTH AND HEMOLYSIS*

INCUBATION IN	MEDIUM	PER CENT GLUCOSE	PNEUMOCOCCUS TYPE 1	β -DIPLOCOCCUS	γ -DIPLOCOCCUS	β -STREPTOCOCCUS
Air	Tryp	0.0	1	0.5b	1.5	2a
	Tryp	0.03	1	1g	2	
	Inf	0.0	1.5	0	1.5	
Candle jar	Tryp	0.0	2	2b	3	4b
	Tryp	0.03	3	2.5b	4	
	Inf	0.0	3	2b	3	

* Growth: 0, none; 1, trace; 2, definite; 3, good; 4, excellent. Hemolysis: a, alpha; b, beta; g, gamma.

Tryp: 2 per cent Tryptose, 0.3 per cent beef extract, 0.5 per cent sodium chloride, 1.5 per cent agar, 0.005 per cent p-amino benzoic acid, pH 7.2.

Inf: beef heart infusion, 1.0 per cent Neopeptone, 0.5 per cent sodium chloride, 1.5 per cent agar, 0.005 per cent p-amino benzoic acid, pH 7.3.

Incubation for 24 hours at 37 C.

equaled the fresh beef infusion base with respect to the support of growth of the test organisms. The small amount of glucose, however, interfered with hemolysis, resulting in the production of a green coloration with some beta-hemolytic streptococci of Lancefield's Group A. This effect was also noted with dextrin but not with glycogen.

Upon increasing the carbon dioxide content of the atmosphere during incubation, the interference with hemolysis by small amounts of glucose was not evident. Comparison of the mediums was therefore made using both ordinary aerobic incubation and incubation in a candle jar. The latter type of incubation not only favored hemolysis but greatly improved the growth of all of the test organisms except *H. influenzae*. This organism appeared not to be influenced by the altered type of atmosphere used during incubation. The advantages of incubation in a candle jar are illustrated in Table 1. Any container that can be made air-tight and possesses an opening sufficiently large to admit a petri dish may be used.

It is evident from Table 1 that 0.03 per cent glucose increases the growth of the test organisms appreciably and that the use of the candle jar further enhances growth and permits the detection of the hemolysis that is obscured by the presence of glucose under ordinary aerobic incubation.

Extensive comparative studies with respect to the support of growth of pure cultures and the primary isolation of the organisms encountered in the diagnostic laboratory, revealed the dehydrated medium (Table 1) enriched with 0.03 per cent glucose to be at least equal to the fresh meat infusion base when incubation in a candle jar was employed.²

II. ENDOWMENT OF BLOOD AGAR WITH THE GROWTH-SUPPORTING PROPERTIES OF CHOCOLATE AGAR

Attention was next directed to the development of a blood agar having the qualities of both blood and chocolate agar. An attempt was made to prepare a combined blood and chocolate agar by adding 5 per cent blood to chocolate agar which had been cleared of coagulated blood by centrifuging. It was found, however, that while cleared chocolate agar supported the growth of *H. influenzae* and *N. gonorrhoeae* very well, the further addition of unheated blood resulted in an improved growth of the *Gonococcus* but an unaccountable inhibition of the growth of *H. influenzae*. This inhibition was found to be due to the removal of the coenzyme (V factor) by the nucleotidase of the erythrocyte. A retarding of this destruction of the coenzyme was obtained by adding 0.1 per cent niacinamide (nicotinamide) to the medium, but the niacinamide was unable to exert continued V-sparing activity at 37 C. and, in addition, inhibited the growth of a few strains of *N. gonorrhoeae*. The maximum amount of niacinamide that would not inhibit these strains was found to be approximately 0.005 per cent. With this amount, the growth of *H. influenzae* although slightly improved, was the same on both the ordinary blood agar and the combined blood and chocolate agar.⁵

With respect to the growth of the *Gonococcus*, it was found that growth was slightly improved by substituting some of the Bacto-Tryptose with Bacto-Proteose Peptone No. 3 and that combined blood and chocolate agar prepared as indicated above, was at least as efficient as the mediums that have been developed expressly for the isolation of this organism.

Although this combined blood and chocolate agar was found to be a suitable single substitute for both blood agar and chocolate agar made from fresh meat infusion, its preparation involved a cumbersome procedure, the centrifuging of the chocolate blood agar. Attempts were made, therefore, to eliminate this step by adding each of the following to the basic medium in addition to the usual 5 per cent blood: yeast extract, blood digests, lysed blood, corn starch and the supernatant from centrifuged heated blood (85 to 90 C. for five minutes).

When added to the medium in one per cent concentration, yeast extract* failed to enhance sufficiently the ability of the blood agar to support the growth

* Prepared by Difco for the enrichment of Hemoglobin-Proteose No. 3 Agar.

of fastidious strains of *N. gonorrhoeae*. The amount of hemolysis produced by some strains of beta-hemolytic streptococci was diminished and with some of these strains an alpha hemolysis was actually obtained. The characteristic morphology of a strain of *Pneumococcus* was markedly altered.

The addition of blood digested with pepsin,⁷ pancreatin and papain was also unsatisfactory in that there was little if any stimulation of the growth of *N. gonorrhoeae*.

By adding 0.25 per cent lysed (frozen and thawed) blood and 0.1 per cent Argo corn starch to the basic agar, it was possible to obtain good growth of the *Gonococcus* with only slight interference with the production of hemolysis. Starch in 0.1 per cent concentration appeared to neutralize the inhibition of hemolysis by the lysed blood and to aid in the enhancement of the growth of the *Gonococcus*. When increased to 0.15 per cent, some interference with hemolysis by a few strains occurred. The resulting medium, however, did not give consistently as good results as did the combined blood and chocolate agar.

The addition of 10 per cent of the supernatants from centrifuged 35 per cent suspensions of chocolate (85 to 90 C.) blood or erythrocytes in water, saline or broth to the blood agar did not appreciably improve the ability of the medium to support the growth of the *Gonococcus*.

In view of the failure to enhance the growth of the *Gonococcus* by enrichment with supernatants from centrifuged suspensions of chocolate blood, it was felt that the beneficial results obtained in the chocolating procedure might be due to factors other than enrichment with blood extractives. The favorable effect of certain carbon preparations on the growth of the *Gonococcus*⁹ and the suggestion by Gould, Kane and Mueller¹⁰ that corn starch adsorbed growth-inhibitory substances from agar indicated that in chocolating blood a similar adsorptive process might be functioning.

Attempts were therefore made to purify two different agar products by adsorption with 1.0 per cent Merck's activated charcoal and then washing. Adsorption of the hydrated agars with charcoal had little effect on the growth of the *Gonococcus*, but subsequent washing of these agars with distilled water did result in marked stimulation of the growth of this organism. The results obtained on ordinary blood agar plates prepared with the washed agars were only slightly inferior to those obtained with the combined blood and chocolate agar.

Using unhydrated granular Bacto-Agar, the effect of thorough washing with distilled water was next investigated. Thirty gm. of the finely granular agar were suspended in four liters of distilled water. The suspension was kept in the refrigerator and stirred two or three times daily and the water renewed every day for five days. The agar was then dried at 90 to 95 C. and 1.5 per cent added to the basic broth. Unwashed agar was used as a control and two kinds of blood agar were prepared from each base, ordinary blood agar and combined blood and chocolate agar. The test organisms were two especially fastidious strains of *N. gonorrhoeae* and three beta-hemolytic streptococci selected because of their tendency to produce alpha hemolysis. The results obtained

after twenty-three hours of incubation in a candle jar are presented in Table 2. It is evident that the Gonococcus-inhibiting factors may be removed from finely ground agar simply by thorough washing with distilled water. This procedure, however, results in a slightly lessened amount of growth of the streptococci.

Since chemical analysis of the agar washings revealed the presence of an appreciable amount of reducing sugar, the effect of adding a small amount of glucose was studied. By increasing the concentration of glucose to 0.05 per cent and with a starch content of 0.1 per cent, the growth of the Streptococcus was restored with no inhibition of beta hemolysis and the medium remained free of the Gonococcus-inhibiting factor. In the preparation of this medium it was noted that the addition of 0.04 per cent lysed blood further stimulated, to a very slight extent, the growth of some strains of *N. gonorrhoeae*. Appreciably larger amounts of lysed blood interfered with the production of beta hemolysis.

TABLE 2
THE EFFECT OF WASHED GRANULAR AGAR ON GROWTH AND HEMOLYSIS*

AGAR	MEDIUM	GONOCOCCUS 1007	GONOCOCCUS 1039	STREPTOCOCCUS 501	STREPTOCOCCUS 727	STREPTOCOCCUS 34
Untreated	Blood	0	0.25	3b	3b	2.5+b
Untreated	Combined	1.5	0.75	3b	2b	2.5+b
Washed	Blood	1.5	0.75	2b	2b	2b
Washed	Combined	1.5	0.75	1.5b	1.5b	2.5b

* Growth: 0, none; 1, trace; 2, definite; 3, good; 4, excellent. Hemolysis: a, alpha; b, beta; g, gamma.

Basic broth: 1.0 per cent Proteose Peptone No. 3, 1.0 per cent Tryptose, 0.3 per cent beef extract, 0.005 per cent niacinamide, 0.005 per cent p-amino benzoic acid, 0.03 per cent glucose, 0.04 per cent corn starch, 0.5 per cent sodium chloride.

Blood: 5.0 per cent to basic medium.

Combined: 3.0 per cent blood to medium heat at 90 C. for five minutes, centrifuge and add 5.0 per cent blood at 45 to 50 C.

The effect of glucose and starch on growth and hemolysis is presented in Table 3. It is evident that: (1) Growth of the Streptococcus varies, up to a certain point, directly with the amount of carbohydrate; (2) For the detection of beta hemolysis by certain streptococci, the presence of starch is necessary; (3) When washed agar and 0.1 per cent starch are used, the glucose may be increased to 0.05 per cent without interference with the production of beta hemolysis; (4) This combination permits streptococcal growth equal to that obtained when unwashed agar and 0.03 per cent glucose are employed; (5) While the addition of starch improved the medium prepared with unwashed agar, as much as 0.15 per cent was not sufficient for the growth of all of the strains of *N. gonorrhoeae* (e.g. #1007); (6) The use of washed agar, 0.05 per cent glucose and 0.1 per cent starch permits the preparation of a blood agar medium that is as efficient as combined blood and chocolate agar with respect to the support of growth of the Gonococcus and, at the same time, favors the surface production of beta hemolysis.

Having succeeded in the development of a medium* that could be easily prepared from a dehydrated base and that possessed the growth-supporting properties of the combined blood and chocolate agar, an experiment was done in order to compare the improved medium with other blood agar bases. Fresh beef infusion base prepared with washed and unwashed agars, Difco's dehydrated Blood Agar Base (beef heart infusion, Bacto-Tryptose, sodium chloride, Bacto-Agar) and BBL's Blood Agar Base (meat infusion, Thiopeptone, sodium chloride, agar) were included.

TABLE 3
THE RÔLE OF GLUCOSE AND STARCH IN GROWTH AND HEMOLYSIS†

AGAR	PER CENT GLUCOSE	PER CENT STARCH	5 PER CENT BLOOD ADDED TO	INCUBATION IN CANDLE JAR FOR 48 HOURS				
				Gonococ- cus 1007	Gonococ- cus 1030	Gonococ- cus 1131	Strepto- coccus 501	Strepto- coccus 727
Washed	0.03	0.0	Ly	3.5	2	2.5	1.5a	2ab
Washed	0.03	0.1	Ly	3.5	1.5	2.5	2b	2b
Washed	0.05	0.0	Ly	3.5	1.5	2.5	2ab	2ba
Washed	0.05	0.1	Ly	3.5	2	2.5	2.5b	2.5b
Washed	0.05	0.15	Ly	3.5	2	2.5	2.5b	2.5b
Unwashed	0.03	0.0	Ly	0	0	0	2.5ab	2+a
Unwashed	0.03	0.1	Ly	0	2	2.5	3.5b	3b
Unwashed	0.03	0.15	Ly	0	2	2.5	3b	3.5b
Unwashed	0.05	0.0	Ly	0	0.5	0	2.5ab	2.5ab
Unwashed	0.05	0.1	Ly	0	2	2.5	2.5ba	2.5ba
Unwashed	0.05	0.15	Ly	0	1.5+	2.5	2.5ab	2.5ab
Unwashed	0.03	0.0	Ch	3.5	2	2.5	2.5a	2a
Washed	0.03	0.0	Ch	3.5	2	2.5	1.5a	1.5a
Washed	0.05	0.1	Ch	3.5	2	2.5	2.5b	2b

† Ly: 0.04 per cent water-lysed blood.

Ch: 3.0 per cent blood heated at 90 C. and removed by centrifuging.

Growth: 0, none; 1, trace; 2, definite; 3, good; 4, excellent.

Hemolysis: a, alpha; b, beta; ab, slight beta; ba, slight alpha.

Medium: 1.0 per cent Proteose Peptone No. 3, 1.0 per cent Tryptose, 0.3 per cent beef extract, 0.005 per cent niacinamide, 0.005 per cent p-amino benzoic acid, 0.5 per cent sodium chloride, 1.5 per cent agar.

* 1.0 per cent Bacto-Proteose Peptone No. 3, 1.0 per cent Bacto-Tryptose, 0.3 per cent Bacto-Beef Extract, 0.5 per cent sodium chloride, 0.005 per cent niacinamide, 0.005 per cent p-aminobenzoic acid, 0.05 per cent glucose, 0.1 per cent Argo corn starch, .14 percent washed agar, cold distilled water. The mixture is stirred to disperse the starch and is dissolved by boiling. The medium is adjusted to pH 7.3 and is autoclaved at 15 pounds steam pressure for fifteen minutes. On cooling to 45 to 50 C. 5.0 per cent human blood and 0.04 per cent water-lysed blood (0.15 per cent of a mixture of one part blood and three parts water) are added. (The addition of this small amount of lysed blood may be omitted if the blood is already slightly lysed because of storage.)

To prepare the washed agar, finely granular agar is washed as a 0.5 per cent suspension in cold distilled water for five days, changing the water daily. The washed agar may be dehydrated with one or two volumes of ethyl alcohol before drying with moderate heat (85 to 100 C.). (Difco's Noble Special Agar has been found to be adequately washed and may be conveniently employed.)

The results obtained after twenty-one hours of incubation in a candle jar are presented in Table 4. The definite superiority of the improved blood agar base over the two dehydrated blood agar bases is evident. Wright's meat infusion medium prepared with washed agar was capable of supporting the growth of *N. gonorrhoeae* but was inferior in this respect to the improved base. Thus, the very simply prepared improved medium was markedly superior to the blood agar bases containing dehydrated meat infusion and was also superior to the very good base prepared with fresh beef infusion.

TABLE 4
COMPARISON OF IMPROVED BASE WITH FRESH MEAT INFUSION AND DEHYDRATED BLOOD AGAR BASES*

MEDIUM	AGAR	BLOOD	GONO- COCCUS 1097	GONO- COCCUS 1030	GONO- COCCUS 1131	STREPTO- COCCUS 301	STREPTO- COCCUS 727	STREPTO- COCCUS 34
Improved base	Washed	5% plus ly	1.5	0.5	0.75	1.5+b	2b	2.5b
Meat Inf base	Washed	5% plus ly	1	0.2	0.5	2b	2b	2.5b
Meat Inf base	Unwashed	5% plus ly	0	0	0	2.5b	2.5b	2.5+b
Difco base		5%	0	0	0	1a	1b	1b
BBL base		5%	0	0	0	1a	1b(tr)	1b

* a, alpha hemolysis; b, beta hemolysis; Ly, 0.04 per cent lysed blood; washed, granular Bacto-Agar washed with water for five days. For other symbols, see legends to Tables 1 to 3.

DISCUSSION

The improved blood agar base has been thoroughly tested in the clinical bacteriologic laboratory. The elimination of the preparation of fresh meat infusion, the use of a single blood-containing medium for the detection of organisms ordinarily encountered in the diagnostic laboratory together with the routine incubation in a candle jar effect a considerable saving in time and effort and, at the same time, a definite improvement in the quality of the bacteriologic work of the clinical laboratory.

In the use of the improved blood agar medium it is important that incubation in a candle jar be employed. The routine use of this type of incubation not only permits the detection of bacteria which require an increased carbon dioxide tension, but also results in better growth of almost all of the other pathogens. This is generally true in the incubation of all blood agars. Of special importance is the fact that the production of beta hemolysis is favored by the atmosphere of the candle jar. As a result of the routine use of this type of incubation with the improved blood agar medium, *N. gonorrhoeae* was isolated on several occasions from specimens in which its presence was not suspected and beta-hemolytic streptococci were found which would have been otherwise missed. Beta-hemolytic streptococci, in almost pure culture, were isolated on several occasions from urethral discharges and prostatic fluid sent to the laboratory for the detection of *N. gonorrhoeae*.

The availability of a medium that will detect beta hemolysis by those beta-hemolytic streptococci which tend to produce a green coloration of blood agar when surface streaking is employed is extremely important in the diagnostic laboratory. Fuller and Maxted⁸ have shown that streptococci that are weakly hemolytic but strong peroxide producers may give green pigment on aerobic blood agar plates. These organisms could be made to produce beta-hemolytic zones of hemolysis by removal of all reducing sugar from the medium, by adding extra catalase or by anaerobic incubation. Such organisms have been found to produce beta hemolysis when streaked on the surface of the improved blood agar medium. The removal of the green coloration may be attributed to the adjusted glucose and starch content of the medium and to the atmosphere provided by the candle jar. (Occasional strains of pneumococci were encountered which showed an appreciably lessened green coloration of the zone of alpha hemolysis.)

The fact that an occasional strain of the *Gonococcus* was inhibited, prevented the use of a sufficient amount of niacinamide for the conservation of the "V" factor. The improved medium, therefore, is only slightly superior to ordinary blood agar for the isolation of *H. influenzae*. This deficiency may be somewhat overcome by inoculating the blood agar plates with a single streak of a *Staphylococcus* culture immediately after the plates have been streaked with the specimen. This use of *Staphylococcus* as a source of "V" factor results in the production of the well known satellite type of growth, making the presence of *H. influenzae* easier to detect.

The mechanism of the inhibition of growth of the *Gonococcus* by unwashed agar* and the possible value of washing the agar in the isolation of other fastidious organisms remain for future study.

SUMMARY

When ordinary aerobic incubation of blood agar is employed, glucose interferes with the production of beta hemolysis. When incubation in a candle jar is employed, the concentration of reducing sugar may be increased appreciably without interfering with the production of beta hemolysis by most beta-hemolytic streptococci. This type of incubation is superior to ordinary aerobic incubation for the isolation of most pathogens on blood agar.

Niacinamide, in concentrations greater than 0.005 per cent, inhibits the growth of some strains of *Neisseria gonorrhoeae*. Only slight stimulation of the growth of *Hemophilus influenzae* is obtained with this amount.

Small amounts of corn starch and lysed blood favor the growth of *N. gonorrhoeae*.

Corn starch neutralizes the inhibition by small amounts of glucose or lysed blood of the production of beta hemolysis.

* By treating agar shreds with methanol, H. L. Ley and J. H. Mueller¹¹ of Harvard Medical School have extracted a *Gonococcus*-inhibiting factor having the properties of a fatty acid. According to H. G. Dunham⁶ of the Difco Laboratories, however, such methanol-extracted agar is slightly toxic for some freshly isolated gonococci.

Factors which inhibit the growth of *N. gonorrhoeae* may be removed from finely ground agar by thorough washing with distilled water.

The use of washed agar and the appropriate proportions of glucose and corn starch permit the simple preparation of a blood agar medium that is superior to a very good fresh beef infusion base and to available dehydrated blood agar bases.

CONCLUSION

A simply prepared blood agar base has been developed which possesses the growth-supporting properties of bases made with fresh beef infusion and of mediums designed expressly for the isolation of *Neisseria gonorrhoeae*. When incubated in a candle jar, it makes possible the use of a single medium for the isolation of organisms ordinarily encountered in the clinical laboratory and for the detection of beta-hemolytic streptococci which give an alpha hemolysis on the surface of other blood agars.

Routine use of this blood agar together with incubation in a candle jar simplifies the work of the clinical bacteriologist considerably and enables the detection of organisms which may otherwise be missed.

Acknowledgments. The author gratefully acknowledges his indebtedness to N. N. Nicholson, Ch. Pharm., USNR, R. C. Holbert, CPhM, USNR, L. Sherman, PhM2c, USNR, and Mrs. L. T. Schermerhorn for their technical assistance.

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ISOLATION OF SHIGELLA FROM THE GALLBLADDER OF A CARRIER*

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Shigellosis is considered primarily an infection of the colon, involving especially the sigmoid and the rectum. Hardy and Watt² stated that the shigellae rarely attack the ileum and they made no mention of involvement of the gallbladder in carriers.

In consulting a number of text books on diseases of the gallbladder^{1, 5, 6, 7} and a system of medicine,⁴ no reference was found to the presence of *Shigella* in the gallbladders of carriers of this organism. It is therefore believed that the recovery of *Shigella* from the gallbladder in a carrier should be reported.

REPORT OF CASE

Clinical data. On November 16, 1938, a white female, 6 years of age, was admitted to the Dixon State Hospital, as an epileptic and classified as an "idiot." The patient, who was apparently physically well developed and able to walk, was nevertheless unable to talk, or to perform any coordinated purposeful movements such as feeding herself. She was unable to exercise voluntary control over the sphincters. On December 21, 1938, she developed diarrhea. On proctoscopic examination, a diagnosis of bacillary dysentery was made and she was treated conservatively. On January 15, 1940, diarrhea was still present and the stools were mixed with blood and mucus. On July 9, she was treated with sulfaguanadine. Although the patient was periodically ill with diarrhea, the diagnosis of shigellosis was first made in October 1943, when *Shigella sonnei* was recovered from a stool specimen sent to the Illinois State Department of Public Health, Division of Laboratories.

On July 1, 1944, at the request of Dr. Warren G. Murray, Superintendent of the Dixon State Hospital, Dr. A. V. Hardy of the United States Public Health Service conducted an intensive study of shigellosis at the hospital. Cultures were routinely taken nearly every day from patients in the Isolation Hospital of the institution.

Methods used. Cultures were taken by the swab method as suggested by Hardy.³ Material thus obtained was immediately streaked on an SS (*Shigella*-*Salmonella*) agar plate. Twenty-four hours later, bacteria from positive colonies (whitish and translucent) were inoculated into Kligler's medium. Colonies of suspected shigellae on culture slants were transferred to the following carbohydrate broths: mannitol, xylose and rhamnose, which served to make the tentative diagnosis. *Shigella paradysenteriae* Flexner produces acid in mannitol but does not cause any reaction or change in xylose or rhamnose. On the other hand, *Sh. ambigua* (*Sh. schmitzii*) ferments rhamnose only, leaving the other two carbohydrates unchanged. *Sh. sonnei*, however, ferments both mannitol and rhamnose and usually does not ferment xylose. The final diagnosis

* Received for publication, December 18, 1946.

is made by serologic study which confirms or disproves the above findings in the sugars.

Study of the carrier state. Since our patient was known to be an intermittent carrier of *Shigella sonnei*, material for culture was taken from her daily, except Sundays and holidays. Table 1 gives the details of the findings on culture beginning July 1, 1944 and ending July 4, 1945, the day of her death. During the year, 329 cultures on Kligler's medium showed growth which was suspicious and of these, 110 or 33.5 per cent were positive for *Shigella sonnei* and 1.0 per cent were positive for *Shigella paradysenteriae* Flexner V. Of these suspicious cultures, 143 or 43 per cent were negative (See Table 1).

TABLE 1

SUMMARY OF DAILY CULTURES OF STOOL OF SHIGELLA CARRIER DURING YEAR JULY, 1944 TO JULY, 1945

DATE	NUMBER OF CULTURES	NEGATIVE	NEGATIVE ON KLIGLER'S MEDIUM	GAS FORMERS	POSITIVE ON KLIGLER'S MEDIUM, NEGATIVE ON SUGARS	<i>Sh. sonnei</i> RECOVERED	<i>Sh. paradysenteriae</i> FLEXNER V RECOVERED	<i>Sh. alcalescens</i> RECOVERED
<i>1944</i>								
July.....	24	4	9	1	2	8		
August.....	29	11	7	2	3	5		1
September.....	37	7	6	7	2	15		
October.....	33	15	8	3		7		
November.....	24	7	2			15		
December.....	27	11	1	2	1	12		
<i>1945</i>								
January.....	30	23			2	5		
February.....	24	18			1	5		
March.....	26	16				10		
April.....	24	10	2	3	1	4	4	
May.....	27	7	6			14		
June.....	23	14				9		
July.....	1					1		
Total.....	329	143	41	18	12	110	4	1
Per cent.....	100.0	43.6	12.5	5.5	3.6	33.5	1.2	0.3

Treatment. During the year the patient was treated intermittently with six courses of sulfadiazine. Each course consisted of one tablet of 0.5 gm. four times daily for four days, the initial dose being double that of the regular dose. The third of these courses was preceded by an intramuscular injection of 1 cc. of typhoid vaccine containing 1.2 billion organisms, to induce hyperpyrexia. The *Shigella* infection was seemingly checked for ten days but the organisms reappeared later. Beginning February 14, 1945, the patient was inoculated with irradiated *Shigella sonnei* vaccine, which contained five billion organisms per cubic centimeter, 2.5 cc. of this vaccine being administered during

the following three weeks. For two weeks following this treatment the organisms were suppressed in the stool. Beginning April 3, the patient was given one tablet of sulfadiazine in combination with two tablets of sulfasuxidine four times daily for six days, without permanent improvement. The urine was checked for sulfa crystals, but no crystals were found at any time.

On July 4, 1945, while the patient was being given an enema for constipation, she suddenly developed continuous epileptic convulsions and expired thirty-five minutes after the beginning of the convulsive seizure.

AUTOPSY FINDINGS

Gross findings. The necropsy revealed the body of a well nourished female, weighing about 68 pounds. The right and left lungs varied in consistence and crepitation. On section of the left lower lobe, the cut surface presented dark purplish areas, differing in consistency. A piece of the dark purple area did not sink in water. The heart weighed 100 gm. The cardiac muscle was pale in color. The kidneys, liver and spleen were slightly congested but normal in appearance.

The gallbladder, which was normal in appearance, was carefully dissected out of the liver and clamped. It was severed distally to the cystic duct and removed from the liver. The wall of the gallbladder was incised with a flamed sterile scalpel. The incision was large enough to admit a sterile applicator. Material obtained on each of two sterile swabs introduced into the gallbladder was streaked, each on a plate of SS agar medium. The gallbladder was then opened and the clear greenish yellow bile flowed freely from it. No gall stones were found.

The gastrointestinal tract was essentially normal in macroscopic appearance, with the exception of enlargement of the mesenteric glands. Since the *Shigella* involves the colon, this part of the intestine was clamped near the end of the ileum just above the ileocecal valve and below, near the end of the sigmoid. Three portions of the colon: ascending, transverse and descending, were distinguished. Each portion of the intestine was incised with a scalpel flamed with an alcohol lamp. The material which was obtained with sterile swabs was then streaked on SS agar plates. Two cultures were taken through each incision. The intestine was then opened longitudinally and inspected for ulcerations but none were found. Sections were taken from the ascending and descending colon for histologic study. The mesenteric glands were markedly enlarged, each being about the size of a small olive. One lymph node was taken for histologic study.

Microscopic findings. The microscopic sections were studied by Dr. B. Lichtenstein of the Psychiatric Institute, Department of Public Welfare, Chicago, Illinois. The intestinal mucosa of the colon was normal in the sections examined. There was slight hyperplasia of the lymphatic areas. The submucosa of the colon and muscularis were hypertrophic. Sections of the lymph node showed marked hyperplasia.

Bacteriologic findings. The cultures taken from the gallbladder were positive for *Sh. ambigua*. On the other hand, cultures from the ascending colon were positive for *Sh. sonnei*.* Negative cultures were obtained from both the transverse and descending colon.

DISCUSSION

During the year of study the patient did not show any positive cultures for *Sh. ambigua*. She may have had an infection with this type of *Shigella* at the beginning of her dysentery in 1939, subsequent to which it lay dormant

* These cultures were verified by the Division of Laboratories, Illinois State Department of Public Health and by the Deutsch Serum Center, Michael Reese Hospital, Chicago.

in the gallbladder. In checking on previous cultures sent to the State Health Laboratory in 1943, only three were positive for *Sh. sonnei*. The finding of *Sh. ambigua* on postmortem cultures would indicate that either the patient had recently contracted it, or else that she had this organism since 1939, when the diagnosis of bacillary dysentery was made by proctoscopic examination. As in typhoid carriers where the gallbladder is the organ of predilection, it is possible that in this patient the gallbladder also was chronically infected with *Shigella* of the Schmitz type.

Another patient, S. Y., female, age 7, in the same room, was a carrier of *Sh. ambigua* during the first eight months of the year. Later, during the last four months, after she contracted infection with *Shigella sonnei*, she was excreting both organisms simultaneously, or either organism intermittently. At the end of this study, however, it was impossible to isolate *Sh. ambigua* from her stools. Apparently, *Sh. sonnei* became predominant over *Sh. ambigua* and perhaps the latter remained dormant in the gallbladder.

In the patient under study, *Sh. sonnei* was isolated from the ascending portion of the colon. The cultures were negative from the transverse and descending portions of the intestine. One would expect to find *Sh. sonnei* predominant in the lower portion of the colon rather than in the ascending.

SUMMARY

The history and autopsy findings are presented of a carrier of *Shigella ambigua* and *Shigella sonnei*. Daily cultures were taken from the stool of this carrier over a period of one year prior to death.

At autopsy, *Sh. ambigua* was recovered from the gallbladder and *Sh. sonnei* was recovered from the ascending colon.

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NEMATODE INFESTATION IN PANGASINAN, LUZON, PHILIPPINE ISLANDS*

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Between February and October 1945, I examined the stools of 550 individuals in Pangasinan, Luzon, Philippine Islands. Because of the unsettled conditions of the period, a good many of these people were from the neighboring province of La Union, and some from elsewhere. Geographical data were not collected. One individual, not included in this series, had a filarial funiculitis, probably acquired on Luzon. Most of the individuals examined were civilians seeking employment with the U. S. Army; a few were hospital patients. Of those examined, 469 were males and 81 were females. The stools were examined by a simple, saturated salt solution flotation, concentration method. In many instances, direct examinations of saline suspensions were also made. The method gave a reliable index of infestation with the common nematodes of the region: *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms (in the few instances in which adult worms were recovered after treatment, they were identified as *Ancylostoma duodenale*). The results are listed in Tables 1 and 2, which show infestation rates of these three nematodes. Two individuals harbored, in addition, larval *Strongyloides stercoralis*, one patient, ova of *Taenia* (species unknown), and one patient, after vermifuge therapy, revealed adult *Enterobius vermicularis*. The low incidence of *Taenia* infestation in this group is undoubtedly due to the small part meat plays in the diet in this region of the Philippines. Infestation with protozoan parasites was not tabulated, for the examinations were incomplete. All stools were not examined directly; zinc sulfate concentration was not used in this group, and the salt flotation method was not effective in demonstrating protozoan parasites. Cysts and trophozoites of various amebae were encountered, however, and in one individual, from whom a history of a severe diarrhea several months previously was obtained, trophozoites of *Balantidium coli* were found in large numbers.

From Table 3, it is seen that 92.6 per cent of all individuals examined harbored nematode parasites. This figure is undoubtedly too low. Of the group of 41 individuals with negative stools, in only 13 were two or more examinations made. If those persons from whom only a single specimen was obtained are omitted from the calculations, the percentage of infested individuals is increased to 97.5 per cent. An estimate can be made of those with one negative stool who would have become positive if subsequent examinations had been made. By applying a correcting factor derived from a study of the number of examinations necessary to obtain a positive examination in those listed as positive (Table 3), the percentage positive for the entire group becomes 95.5 per cent.

Examination of Table 1 brings out an interesting fact. While there is no significant difference between the infestation rates of men and women with *Ascaris* or

* Received for publication, November 21, 1946.

Trichuris, there is a striking difference in the hookworm infestation rate. That this difference is far too great to be explained as a sampling error is shown by the calculation of the Chi square value.

There are, undoubtedly, several factors which explain the difference in infestation rates in the three common nematodes. In all probability, all of them are related to the different modes of infestation. *Ascaris* and *Trichuris* both obtain

TABLE 1

INCIDENCE OF ASCARIS, TRICHURIS AND HOOKWORM INFESTATION IN 550 FILIPINOS

	MALE SUBJECTS		FEMALE SUBJECTS		TOTAL		CHI SQUARE χ^2	PROBABILITY p
	Number	Per cent σ	Number	Per cent σ	Number	Per cent σ		
Total examined.....	469		81		550			
Total positive.....	433	92.3 ± 1.25	76	93.8 ± 7.63	509	92.6 ± 3.52	0.23	0.63
<i>Ascaris</i>	343	73.2 ± 2.05	65	80.3 ± 4.42	408	74.4 ± 1.86	1.82	0.18
<i>Trichuris</i>	198	42.4 ± 2.28	36	44.4 ± 5.52	234	42.6 ± 2.11	0.14	0.70
Hookworm.....	235	50.1 ± 2.31	10	12.3 ± 4.10	245	44.6 ± 2.12	39.1	2×10^{-9}

TABLE 2

ANALYSIS OF INCIDENCE OF NEMATODE OVA (AND/OR PARASITES) IN THE STOOLS OF 550 FILIPINOS

NEMATODE	MALE SUBJECTS		FEMALE SUBJECTS		TOTAL		CHI SQUARE χ^2	PROBABILITY p
	Number	Per cent	Number	Per cent	Number	Per cent		
<i>Ascaris</i> *.....	110*	23.5	36	44.4	146	26.6		
<i>Trichuris</i>	22	4.7	9	11.1	31	5.6		
Hookworm.....	51	10.9	1	1.2	52	9.5		
<i>Ascaris</i> and <i>Trichuris</i>	66	14.1	21	25.9	87	15.8		
Hookworm and <i>Ascaris</i> †.....	74†	15.8	3	3.7	77	14.0		
<i>Trichuris</i> and hookworm.....	17	3.6	1	1.2	18	3.3		
<i>Ascaris</i> , <i>Trichuris</i> and hookworm‡.....	93‡	19.8	5	6.2	98	17.8		
Single infestation.....	183	38.9	46	56.8	229	41.7	4.74	0.03
Multiple infestation.....	250	53.3	30	37.0	280	50.9	7.31	0.0069
Total infestation.....	433	92.3	76	93.8	509	92.6	0.23	0.63

* Includes one case with *Tacnia* infestation also.

† Includes one case with *Enterobius vermicularis* also.

‡ Includes two cases with *Strongyloides stercoralis* also.

entrance to the human body by way of the digestive tract; the hookworm, in most instances, gains entrance through the skin.

The groups of men and women were not entirely similar in composition. The men were of wider age distribution and included many individuals of the field laborer class. A large proportion were "permanently" shoe-less, and undoubtedly spent much time in the fields and rice paddies. The females were mostly young girls, of better education. They included school girls, teachers,

nurses, clerks and minor civil servants. Almost all of them wore shoes, sabots, or the native woven sandal. It is improbable that more than a few were field workers.

From Table 1, it is seen that there is little probability that the difference in infestation in males and females with *Ascaris* or *Trichuris*, or in nematode infestation in general, is other than a sampling error. On the other hand, the dif-

TABLE 3
RESULTS OF STOOL EXAMINATIONS IN RELATION TO POSITIVE FINDINGS

NUMBER OF SUBJECTS	NUMBER	PER CENT σ
Number with negative examinations	41	
1 negative examination.....	28	
2 negative examinations.....	11	
3 negative examinations.....	1	
4 negative examinations.....	1	
Number with positive findings	509	92.6 \pm 3.52
Positive		
On first examination.....	488	
On second examination.....	15	
On third examination.....	4	
On fourth examination.....	2	
Corrected total positive examinations (see text).....	524	95.5 \pm 2.80

TABLE 4
INCIDENCE OF OVA AND PARASITES IN STOOLS OF 140 AMERICAN SOLDIERS

	NUMBER	PER CENT σ
Individuals examined.....	140	100
Individuals infested.....	21	15.0 \pm 3.02
With <i>Ascaris</i>	14	10.0 \pm 2.54
With <i>Trichuris</i>	5	3.7 \pm 1.60
With <i>Ascaris</i> and <i>Trichuris</i>	1	0.71 \pm 0.70
With <i>Strongyloides</i>	1	0.71 \pm 0.70
Individuals harboring cysts of <i>E. histolytica</i> *.....	5	3.7 \pm 1.60

* Zinc sulfate centrifugation-flotation method.

ference in hookworm infestation rate is significant, there being less than two chances in 1,000,000,000 that the difference between infestation in males and females could have arisen by mere sampling. This significant difference is probably explained by the difference in exposure. Hookworm infestation is largely due to the exposure of the bare skin to the larval parasite in the polluted fields and rice paddies. Both groups are the victims of the general low level of sani-

tation, which explains the equally very high incidence of water, food and dust borne helminthic infestation. It will be noted in Table 2 that total infestation rates are similar in males and females but that the rate of multiple infestation, which might be related in some way to the degree of personal sanitation, is significantly lower in females than in males. No attempt was made to express quantitatively the degrees of infestation. The impression was obtained, however, that persons with multiple infestation were more heavily infested, and that heavy infestations were rare in females. It will be seen in Table 2 that single infestations were somewhat more frequent in females, although the statistical probability is not high.

For comparison, it is interesting to note the incidence of infestation in a small group of men introduced into the same environment from a more hygienic one. These men were well shod and armed with a modicum of sanitary training, and their work did not expose them to the hazards of the open fields and rice paddies.

Before they arrived in the Philippines, a survey revealed that less than 2 per cent of the men had hookworm infestation, all of these being from the southern part of the United States. No other infestations were discovered, although there might have been some in the latent phase, acquired during a six months' stay in New Guinea. In the latter area, however, hookworm was the predominant helminth and *Ascaris* and *Trichuris* were rare. During approximately six months' exposure on Luzon, 15 per cent acquired helminthic infestations. *Ascaris* and *Trichuris* were numerous, but hookworm infestation did not occur. These results are tabulated in Table 4. The infestation rate is much lower than in the civilian groups, but was steadily rising during the period of exposure. Probably differences in sanitation would have prevented the rate reaching that of the civilian population, but certain individuals were obviously breaking sanitary controls. How far individual and small group control methods can be effective in a highly contaminated area is problematic.

SUMMARY AND CONCLUSIONS

Data are presented of infestation rates in Pangasinan, Luzon, Philippine Islands, for the common nematode parasites in (1) an indigenous group and, (2) a group of individuals introduced from the United States.

Significant differences in the rate of hookworm infestation in males and females were found. Infestation rates of *Ascaris lumbricoides* and *Trichuris trichiura* are essentially the same in male and female persons. These differences are related to the differences in the modes of infestation and to the differences in type of exposure of males and females.

Acknowledgments. I wish to express my thanks to Mrs. Raymunda Herrera, my secretary, who made it possible to distinguish names of male and female persons. Most of the stool preparations were made by James Estridge.

USE OF Rh-NEGATIVE MOTHER'S BLOOD IN TRANSFUSIONS IN NEWBORN BABIES*

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It was recently suggested by Mayes¹ that administration of blood by way of the umbilical cord at birth from an Rh-negative mother to her baby was helpful in the case of erythroblastosis. The results of 78 such cord transfusions were presented as evidence in favor of the hypothesis. However, since the idea is contrary to the accepted mechanism of action of the Rh factor, it appears pertinent to examine these data in more detail. The mother's blood should contain, if they are present at all, the anti-Rh agglutinins which are responsible for the damage to the baby's blood cells, and should, therefore, be unsafe to use. Also, these data lend themselves to an analysis of the type which Snyder² has made, which should be of interest to both the geneticist and the physician.

In the report by Mayes of the 78 babies who were delivered from Rh-negative mothers and given cord transfusions from the mothers, three showed erythroblastosis and two died (both premature babies in whom the diagnosis of erythroblastosis was not made). Thus, approximately 6.5 per cent of these deliveries resulted in erythroblastosis or death. This figure is considered by Mayes to be low and is interpreted by him to indicate that the "mother's blood seems to agree with the babies." In order to evaluate the results, an attempt is here made to analyze the 78 cases in terms of what would be expected in the absence of a transfusion and to determine what proportion of the infants would be expected to develop erythroblastosis under the conditions that were present.

It has been realized for some time that an Rh-negative mother can usually safely deliver her first Rh-positive child since she has not had an opportunity previously to build up antibodies against the Rh factor. Mayes stated that 37 of the 78 mothers under consideration were primiparas (47.4 per cent). Therefore, only 41 of the mothers would be expected to have developed enough antibody to produce an erythroblastotic baby.

In order for the fetus to cause the Rh-negative mother to build up agglutinins against the Rh factor, the fetus must have the agglutinogens; i.e., be Rh-positive. If the father and mother are both Rh-negative, the fetus will also be Rh-negative. Mayes was unable to test all of the fathers, but of the 47 tested, six were found to be Rh-negative. Therefore, 6 of the original 78 can be dismissed as not being expected to develop erythroblastosis. Of 78 fathers, however, we would expect to find closer to 10 Rh-negative, since 13 per cent of the population are Rh-negative.² So, if all Rh-negative fathers are considered, the number of babies that might be expected to develop erythroblastosis is decreased.

About 46 per cent of the population is heterozygous for the Rh-negative

* Received for publication, December 2, 1946.

gene, rh^2 . Half of the children of the heterozygous ($Rh\ rh$) man married to an Rh -negative woman ($rh\ rh$) would be expected to be negative. These negative children cannot develop erythroblastosis. About 36 of the 78 fathers would be expected to be heterozygous and, therefore, about 18 of their children should be Rh -negative, thus giving a total of 28 Rh -negative children, or 36 per cent. Mayes tested 61 of the 78 babies and found that 31 per cent of them were negative. That is in fairly close agreement with the expected incidence.

Considering both factors, first born and Rh -negative children, we can calculate the expected number of children that might be normal for one or both of these reasons. Since 47.4 per cent of the children were first born and 36 per cent are expected to be Rh -negative, approximately 33.7 per cent should remain with the conditions necessary to develop erythroblastosis (52.6 per cent not first born \times 64 per cent Rh -positive children). That is to say that in only 26 of the 78 births was there an Rh incompatibility.

Also, some of the 26 children who were not first born, but were Rh -positive, would be expected to have older siblings who were negative if the father were heterozygous, *i.e.*, some of these 26 would be the first Rh -positive even though not the first born. Snyder calculated by appropriate statistical methods of analysis of gene frequency that Rh -negative women will marry heterozygous men about 15 per cent of the time. On calculating the chance, it is found that of 26 mothers, each giving birth to two children, one mother may be expected to have the first child negative and the second positive.

This still leaves 25 children who were born under circumstances expected to lead to erythroblastosis, or one-third of those children born to Rh -negative mothers. It is of interest to compare this result to Snyder's calculation made by a statistical analysis of gene-frequency in which he obtained an expected finding of one in 17 of all births. The figure derived from Mayes' data is one in 27 (since Rh -negative women are married to Rh -positive men in only 11.3 per cent of all matings, one-third of 11.3 per cent is 3.7 per cent, or one in 27 of all births may be expected to show the effect of the immunization of the mother by the Rh factor). This expected figure is considerably less than the theoretical expected finding of Snyder. The reason may probably be found in the large number of first born children in Mayes' data (47.4 per cent) as compared to the total American population in which the first born comprise only about 31 per cent of all births.

As Snyder pointed out, even though only one in 17 of all births may be expected to result in erythroblastosis, actually only one in about 200 does. He suggested that it may be that only some women are capable of producing potent antibodies against the Rh agglutinogens. In other words, actual results show that of the children who should develop erythroblastosis, only about 10 per cent do. Therefore, according to the data of Mayes, of the 25 cases which should develop erythroblastosis, it would be expected that only 10 per cent of children (2 or 3) would actually show symptoms. This, in fact, did occur. In 78 births, in instances in which the mother was Rh -negative, 3 babies had a diagnosis of erythroblastosis. In addition, there were two deaths in which no diagnosis was made.

It may be concluded at this point that regardless of the transfusion of mother's blood to the child, the number of cases of erythroblastosis was the same as would be expected. The transfusion does not appear either to forestall the erythroblastosis or to increase its occurrence. In a control group where no transfusion was given, Mayes found in 59 deliveries that five resulted in erythroblastosis and that two of these died. This means approximately 8.5 per cent of these deliveries resulted in erythroblastosis or death as compared to 6.5 per cent in the group receiving a cord transfusion. There is not a significant difference between the two figures. However, one should not expect, *a priori*, the transfusion of mother's blood to increase the frequency of erythroblastosis, for if the mother's blood lacks a high titer of the anti-Rh agglutinins, it should be as useful in a transfusion as any blood; if her blood does have a high titer it would probably harm the fetus regardless of the transfusion. Nevertheless, it seems plausible that a transfusion, using blood with a high titer of the anti-Rh agglutinins, should increase the symptoms of erythroblastosis in the baby even though the symptoms are initiated in the fetal stages. Therefore, an examination of the affected babies appears pertinent.

As has been stated, two babies died. It is not known if they were erythroblastotic. Both of these infants were premature, one having a gestational period of 34 weeks, the other 31 weeks. The first of these weighed 4 pounds, 15 ounces. According to Mayes' data, 11.5 per cent of such babies die. The other one weighed 3 pounds, 9 ounces, and 45.4 per cent of babies of this weight die. No conclusion can be reached as to the influence of the transfused mother's blood in either case. The first of the babies which definitely developed erythroblastosis was pronounced as being in "fair condition" at birth. It was a full term child weighing 7 pounds, 3½ ounces. Soon after the cord transfusion of mother's blood, symptoms of erythroblastosis developed. On the following day, the baby was given a transfusion from a professional Rh-negative donor. The baby recovered. Two other infants were diagnosed as erythroblastotic and both recovered.

Mayes furnished several additional cases not included in the original 78 cases. One of these was a baby who became jaundiced soon after birth. It was given a transfusion of Rh-negative mother's blood on the second day. The baby showed improvement until the eighth day. On the fourteenth day it was given blood from a professional Rh-negative donor and recovered. In this instance, if the mother's blood had a detrimental effect, the effect was delayed for several days. In another case, the baby was given a cord transfusion of Rh-negative mother's blood, and was markedly jaundiced on the day of the delivery. On the following day, a transfusion was given by a professional Rh-negative donor. The baby recovered. In another case, an Rh-negative mother's blood was transfused to her two and one-half hour old baby. The baby died on the third day. The same mother bore another baby four years later which was jaundiced. This time the blood of an Rh-negative donor was given and the baby recovered.

These cases do not support the idea that a transfusion of mother's blood is helpful. In all but two of these nine cases, the recovery could be attributed

to the transfusion from a professional Rh-negative donor. In the two exceptional cases which recovered, Mayes did not state whether they received subsequent transfusions or not. Therefore, no conclusive evidence can be drawn from these data to demonstrate that transfusions to Rh-positive babies of Rh-negative mother's blood have a harmful effect, although a harmful effect is indicated. It is shown, however, that erythroblastosis is not forestalled by such a transfusion, nor should it be in light of the present knowledge of the genetics and physiology of the Rh factor.

SUMMARY

The advisability of giving mother's blood which is Rh-negative to newborn babies is critically questioned. It is concluded from an analysis of data that such transfusions do not lower the occurrence of erythroblastosis among the babies and, in some cases, may provoke or aggravate the condition.

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GRANULAR CELL MYOBLASTOMA (MYOBLASTIC MYOMA, RHABDOMYOME GRANULO-CELLULAIRE)*

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In 1926, Abrikossoff¹ described a series of five tumors (three from the tongue, one from the upper lip and one from the depths of the gastrocnemius muscle) which he interpreted as arising from myoblasts. These tumors were composed of fairly large cells with small, single or multiple, pyknotic nuclei and characteristically contained large amounts of coarsely granular cytoplasm, much of which was eosinophilic. To these tumors he gave the name myoblastic myomas. In two of them, the cells comprising the tumor were typical unstriated myoblasts, while in the other three, both longitudinal and cross striations were found in variable amounts. Abrikossoff¹ was of the opinion that these tumors arose from neighboring striated muscle elements following degeneration, trauma, or inflammation. This concept of the pathogenesis of these tumors was somewhat modified following the report of five additional cases by Klinge¹⁷ in which he showed that similar tumors arose in situations in which striated muscle was not ordinarily present. Abrikossoff² subsequently agreed that by dysontogenetic inclusions of primitive myoblasts in various situations these tumors might arise anywhere and, indeed, this has proved to be the case.

Following Abrikossoff's original report,¹ a considerable number of instances of myoblastic myoma has appeared in the literature. In 1944, Howe and Warren¹⁴ reported 10 cases and reviewed the existing literature on the subject and Crane and Tremblay,⁶ in 1945, were able to collect 157 cases to which they added five. Within the past several years, these tumors have gained additional significance by reason of their gross similarity to carcinoma, when they appear in the breast, and also by reason of recent reports of malignant changes arising in some of them. In the past two years, I have seen five cases of so-called myoma with an additional case from old material which had been previously undiagnosed.

REPORT OF CASES

Case 1

Clinical data. The patient was a 60 year old, white male who noted a painless lump in his right breast one year before admission. He stated that the lump was not as freely movable then as it was at the time of admission, and that it had not increased in size. Upon examination, a hard, painless, freely movable mass the size of a large walnut was palpated in the upper, inner quadrant of the right breast. The skin was not adherent to it and no discharge from the nipple was present. The tumor was removed; frozen section was performed and a diagnosis of benign tumor was made.

Gross description. The tumor lay in fat, was roughly globular, firm, and sharply outlined, but not encapsulated and measured 2 by 2.5 cm. The cut surface presented a yellowish white, finely granular and slightly lobulated appearance with minute, pearly gray foci. It was strikingly avascular.

* Received for publication, December 24, 1946.

Microscopic description. Sections revealed an encapsulated nodule lying in subcutaneous fat. The capsule was made up of loose and edematous fibrous connective tissue which extended into the tumor in thick and thin septa. The tumor itself was composed of large and small groups of cells which were separated from each other by irregular septa having only an occasional blood vessel. From the connective tissue septa, fine but quite regular fibers penetrated into the groups of cells. The cells composing the tumor were roughly polyhedral in shape and contained abundant granular cytoplasm which took the eosin stain. In many situations no cell outlines could be seen and the tumor appeared to form a syncytium. The cells contained small, dark-staining nuclei which varied only slightly in size, shape and staining quality and occasionally contained small nucleoli. Many of the cells failed to show nuclei at the level of the section and within a few of the cells several nuclei were encountered. The majority of the cells appeared to be cut in cross section and the impression was gained that they were somewhat elongated. Careful search failed to reveal any transverse striations in any of the cells cut tangentially. No vacuoles were seen in the cytoplasm. Frozen sections stained for fat failed to show any lipoids within the tumor cells. Sections stained by Best's carmine method failed to reveal glycogen. Van Gieson's stain showed tumor cells that took an atypical orange color. This tumor was removed in 1936 and the diagnosis of myoblastoma was established in 1939.

This is an instance of granular cell myoblastoma occurring in a situation in which no skeletal muscle is ordinarily present. The tumor grossly simulated carcinoma but was encapsulated. The patient is alive and well, ten years after removal of the tumor, and has had no recurrence to date.

Case 2

Clinical data. The patient was a 49 year old white female who had a small, slightly elevated, nonpainful nodule on the left lateral aspect of the tongue for a number of years which had not recently increased in size. No further history was available. The nodule was excised.

Gross description. The specimen consisted of a fragment of tissue about 1 cm. square, covered on one surface by an intact layer of epithelium. Upon section, the tissue was gray in color.

Microscopic description. Section of tissue submitted for biopsy showed a fragment of tongue covered on one surface by slightly thickened keratinized stratified squamous epithelium showing slight parakeratosis. The epithelial pegs were somewhat broadened but the basilar layer was sharply defined. Beneath the epithelium, a few small collections of round cells were noted. The bulk of the subepithelial tissue was composed of rather irregular anastomosing bundles of cells which were relatively large, showed both centrally and peripherally placed nuclei and contained abundant, distinctly granular, pale-staining cytoplasm. Between these groups of cells normal striated skeletal muscle fibers were seen. In some situations, muscle fibers appeared to be continuous with these granular cells, but this was more apparent than real and represented overlapping of the granular cells by striated muscle fibers. In one situation, a transition from a striated muscle fiber to a granular cell was noted which lay in the same optical plane. The granular cells extended up to the epithelium and extended downward to the limit of the section. The nuclei of these granular cells were for the most part pyknotic and no mitotic figures were present. The supporting tissue between the granular cells consisted of both coarse and fine fibrils and many groups of cells appeared to have no surrounding, supporting fibrils.

This is an instance of a typical myoblastoma occurring in a situation in which skeletal muscle is ordinarily encountered. It showed the usual intimate admixture of granular cells and skeletal muscle fibers seen in these situations.

Actual transition in the same plane of section between striated muscle fibers and granular cells could be identified in one area. No linear arrangement of fibrils or granules was present. As far as can be ascertained, this patient has had no recurrence.

Case 3

Clinical data. The patient was a 31 year old white male who noticed a lump in his abdominal wall about one year before admission to the hospital. He stated that it had grown gradually and that lately it had become painful. Physical examination revealed a firm, nontender nodule, measuring 2.5 by 2.5 inches, in the left lower quadrant of the abdominal wall. The skin was intact over the nodule; there was no discoloration and the tumor seemed to be movable over the abdominal muscles. The operative note stated that the nodule was situated just medial to the left iliac crest and at the level of the crest. The nodule was excised and was found to be adherent to the fascia of the external oblique muscle but could be separated from that fascia.

Gross description. The specimen consisted of a roughly oval mass of fairly firm and encapsulated tissue which measured 4 by 2.5 by 3.5 cm. This mass was surrounded by a small amount of fibroadipose tissue, was slightly lobulated, cut with slight resistance and, on cut section, was gray in color and homogeneous in appearance.

Microscopic description. Two sections revealed a poorly encapsulated mass of tissue surrounded by fat. The tissue was composed of large, poorly defined, cellular structures which had a tendency to form an irregular syncytial mass. These cells contained abundant distinctly granular, eosinophilic cytoplasm and rather small and densely-staining nuclei which, at times, lay in the center of the cells and occasionally near the periphery. In many cells the granular cytoplasm presented a spider-like arrangement. Between the syncytial masses of cells, numerous fine and, at times, coarse connective tissue strands divided the mass into irregular alveoli. Careful search failed to reveal any cells in which transverse striations could be identified. No skeletal muscle fibers were present. At the periphery of the tumor mass, small focal collections of lymphocytes were occasionally seen. The capsule was thin and fibrous and the adjacent fat appeared normal. Sections stained by the Masson trichrome method revealed that the large, granular cells took a red stain, suggesting that they were muscle cells, and frozen sections stained by sudan III showed no fat within the large, granular cells.

This encapsulated tumor occurred in a situation where skeletal muscle is ordinarily not encountered and no connection with the underlying abdominal muscles could be demonstrated. The granular cells, however, were identical with those encountered in situations where these tumors are intimately associated with skeletal muscle. There has been no recurrence in almost two years.

Case 4

Clinical data. The patient was a 48 year old white housewife who had noted a small painless nodule on the lateral aspect of the tongue. The duration of this growth was not known and there was no history of recent increase in size. On physical examination, a small, nodule, firm in consistency and covered by intact mucous membrane was noted on the left lateral aspect of the mid-portion of the tongue. It was thought to be a small fibroma and was excised.

Gross description. The specimen consisted of a small, roughly oval fragment of pale pinkish gray tissue, measuring 6 mm. in diameter. One surface was covered by slightly roughened mucous membrane and the underlying nodule was gray in color and sharply circumscribed.

Microscopic description. The section revealed an oval fragment of tissue which was covered on one surface by a slightly thickened layer of stratified squamous epithelium, the basal layer of which was sharply defined. The underlying tissue was composed of aggregates of large, circular to oval cells containing abundant coarsely granular and somewhat eosinophilic cytoplasm. These cells contained single, and at times, multiple, small oval-shaped nuclei some of which contained prominent nucleoli. The position of the nuclei in the cells was inconstant. The tumor was moderately well circumscribed, but not encapsulated, and was irregularly surrounded by masses of skeletal muscle, some of the fibers of which penetrated into the tumor nodule and some of which lay deeply embedded among the granular tumor cells. In many places, these transversely striated skeletal muscle fibers overlay granular cells and, at first glance, the granular cells appeared to be continuations of the skeletal muscle fibers. Examination under oil immersion showed that the majority of these striated muscle cells lay in a different plane from the underlying granular cells. Only occasionally could obvious transitions from striated muscular fibers to granular cells be identified as lying in the same plane. The granular tumor cells were supported upon thin fibrous connective tissue septa which, in many places, sent delicate fibrils to envelop individual cells but, not infrequently, several cells lay in apposition to each other without obvious supporting stroma. No vacuoles were present in any of the cells and the nuclei were quite regular in size, shape and staining quality. The granular cells appeared to extend up to the limits of removal.

This case is an instance of granular cell myoblastoma arising in a situation where skeletal muscle ordinarily occurs. It showed an intimate admixture of granular cells and skeletal muscle fibers and, in several places, obvious transitions from transversely striated muscle fibers to granular cells could be identified. The transverse striations at the point of transition were immediately lost and no specific longitudinal arrangement of granules could be seen. Although there was a doubt as to the completeness of removal of this tumor from a microscopic point of view, no recurrences have been noted in two years.

Case 5

Clinical data. The patient was a 41 year old white male who had noted a slightly elevated, slate colored and nonpainful nodule on the lateral aspect of the left thigh for the past two years. There was no history of a recent increase in size.

Gross description. The specimen consisted of an elliptical fragment of skin and subcutaneous tissue, measuring 3 by 1.5 cm. The skin was slightly wrinkled and a few hairs protruded from the surface. In the center, a firm, slightly elevated nodule, measuring 0.7 by 1 cm., was present. This nodule was covered by a layer of thin, gray skin which appeared to be devoid of hair. On section, this nodule presented a homogeneous, yellowish gray cut surface which could be distinguished with difficulty from the underlying adjacent dermis.

Microscopic description. The section revealed a thin, elongated strip of skin which was covered on one surface by a thin layer of stratified squamous epithelium, the basal layer of which was sharply defined. The dermis was quite fibrous and contained a few scattered hair follicles and a few sweat glands. In the central portion of the specimen and in the upper portion of the dermis, aggregates of large, oval cells were seen containing large amounts of coarsely and finely granular eosinophilic cytoplasm. The cytoplasm was not vacuolated. The nuclei of these cells were small and oval in shape and contained single nucleoli. The cells within these groups lay in apposition to each other and were surrounded by rather compact fibrous connective tissue envelopes which occasionally sent fine fibrils into the cell masses. No skeletal muscle fibers were present and the groups of granular cells were relatively few in number. The lesion was rather diffuse and not circumscribed.

This is an instance of granular cell myoblastoma occurring in a situation in which skeletal muscle is not normally encountered. The cells comprising the tumor were identical with those seen in situations where these tumors arise in skeletal muscle. The lesion in this case was quite small, fibrous and diffuse. There has been no recurrence within the past one and one-half years.

Case 6

Clinical data. The patient was a 50 year old white housewife who had suffered from hemorrhoids for twenty years. About four years before admission, she first noticed what she described as a "seed" on one side of the anus. It became larger and painful to touch and contact. On physical examination, a nodule about the size of a filbert was noted at the 3 o'clock position in the anus. This was regarded as a thrombosed hemorrhoid.

Gross description. In the largest hemorrhoidal tag immediately underneath the epidermis, a sharply circumscribed but nonencapsulated nodule measuring 1.2 cm. was noted. It was roughly spherical in shape, firm in consistency and, on section, revealed a yellowish gray, homogeneous surface in which no lobulation or granularity was noted.

Microscopic description. The largest fragment showed a well circumscribed, nonencapsulated tumor nodule composed of fairly large, circular cells which contained abundant fine and coarsely granular, eosinophilic cytoplasm. These cells contained single and, at times, multiple nuclei which were uniform in appearance, round to oval in shape and contained a single prominent nucleolus. The tumor cells were supported upon thin, delicate connective tissue strands which, in some places, gave a suggestive alveolar pattern to the tumor. Some of these delicate strands surrounded individual cells while other tumor cells lay in apposition to each other without supporting stroma. At the base of the tumor, a number of strands of skeletal muscle were encountered which penetrated into the tumor itself and here showed an admixture of striated skeletal muscle fibers and granular tumor cells. Careful search failed to reveal any transverse striations in any of the granular tumor cells. In the center of the tumor, a small collection of epithelial cells was noted. These were of basal type, were uniform in appearance and probably represented an epithelial peg extending rather deeply and cut tangentially. Near the base of the tumor, a portion of sweat gland was partly surrounded by granular tumor cells.

This case showed typical granular cells and a slight admixture of skeletal muscle fibers but no transitional forms between granular cells and striated muscle fibers could be seen.

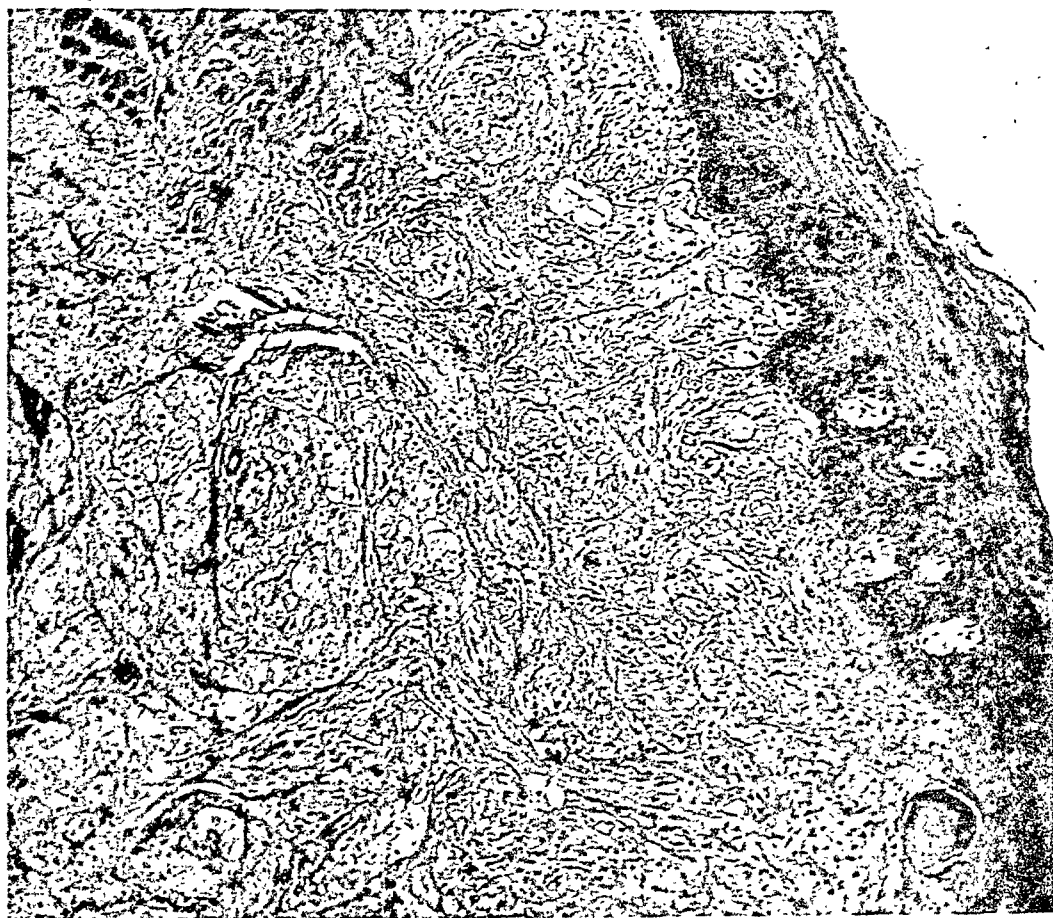
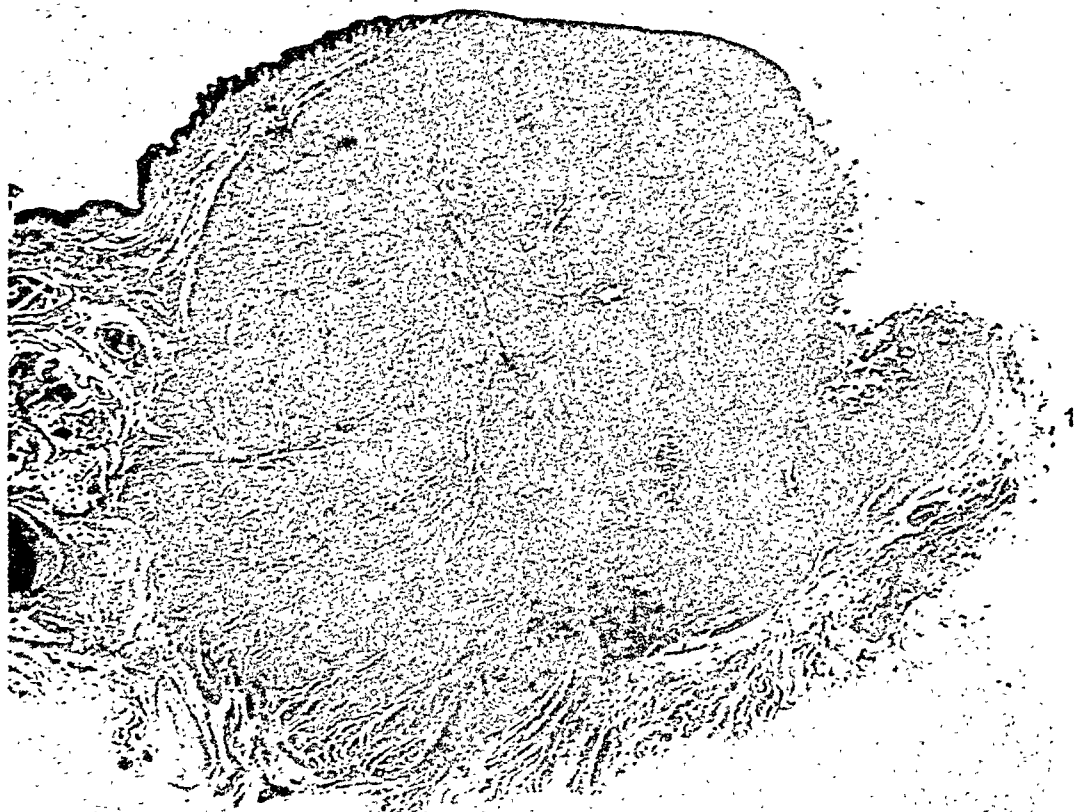
PATHOLOGIC CHARACTERISTICS

The present series of tumors does not differ in any essential respects from those previously recorded in the literature.

While these tumors may appear anywhere in the body and in a person of any age group, by far the largest number occur in the tongue. Crane and Tremblay⁶ stated that 38 per cent arose in this location. The tumors have been reported

FIG. 1. LOW POWER VIEW OF GRANULAR CELL MYOBLASTOMA OF ANUS, CASE 6. Note admixture of skeletal muscle fibers at base, and thrombosed hemorrhoidal vein at left. The defect in the epidermis (upper right) is an artefact. Masson trichrome. $\times 4$.

FIG. 2. GRANULAR CELL MYOBLASTOMA OF TONGUE, CASE 4, SHOWING CHARACTER AND ARRANGEMENT OF TYPICAL GRANULAR CELLS AND RELATION TO OVERLYING EPITHELIUM. Note the admixture of skeletal muscle fibers in upper left hand corner. Masson trichrome. $\times 92$.



in the following situations, in order of decreasing frequency: tongue, skin, subcutaneous tissue, muscle, maxilla, larynx and vocal cord, breast, mandible, lip, trachea and bronchi, ear, alveolar process and anus, with single cases in bladder, esophagus, lacrimal sac, floor of mouth, pharynx, spermatic cord, urethra and vulva. It is at once obvious that these tumors frequently arise in situations where skeletal muscle ordinarily does not occur.

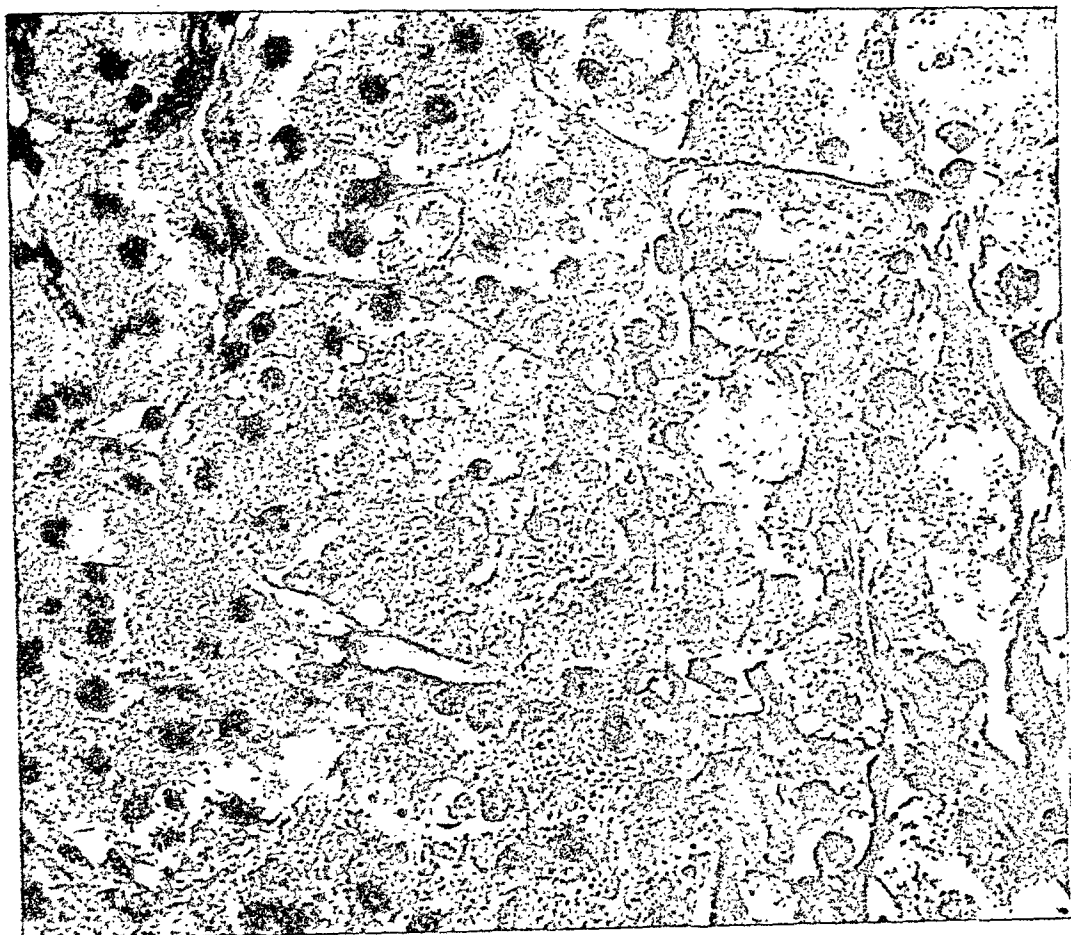
Grossly, these tumors are small, usually from 0.5 to 2.0 cm. in diameter, but tumors up to 10 cm. in diameter have been recorded. The tumors are generally sharply circumscribed and may even be encapsulated, but instances of poorly defined lesions may occur. The cut surfaces vary from yellowish gray to pearly white in color and may present a finely nodular or finely lobulated appearance. They are usually elevated above the skin or mucous membrane but in certain situations, as in the breast, may be more deeply placed. Pedunculated and polypoid tumors have been observed.

Microscopically, the tumors are composed of characteristic cells which are large, varying from 20 to 60 microns in diameter, are usually polyhedral, but may be cylindrical or ribbon-like in shape, and contain abundant coarsely granular acidophilic cytoplasm. In some of the cylindrical or ribbon-like cells, apparent transverse striations can sometimes be seen, but this is inconstant and rare. The relation of striations to these granular cells will be mentioned later. The nuclei of these cells are oval in shape, varying from 7 to 10 microns in diameter, are vesicular and usually contain one prominent nucleolus. At times the nuclei are small and pyknotic. The position of the nucleus in the cell is variable and cells which are apparently single may show multiple small pyknotic nuclei. Mitotic figures were not noted in the present cases but have been observed by others (Ceelen⁴ and Derman and Golbert⁷).

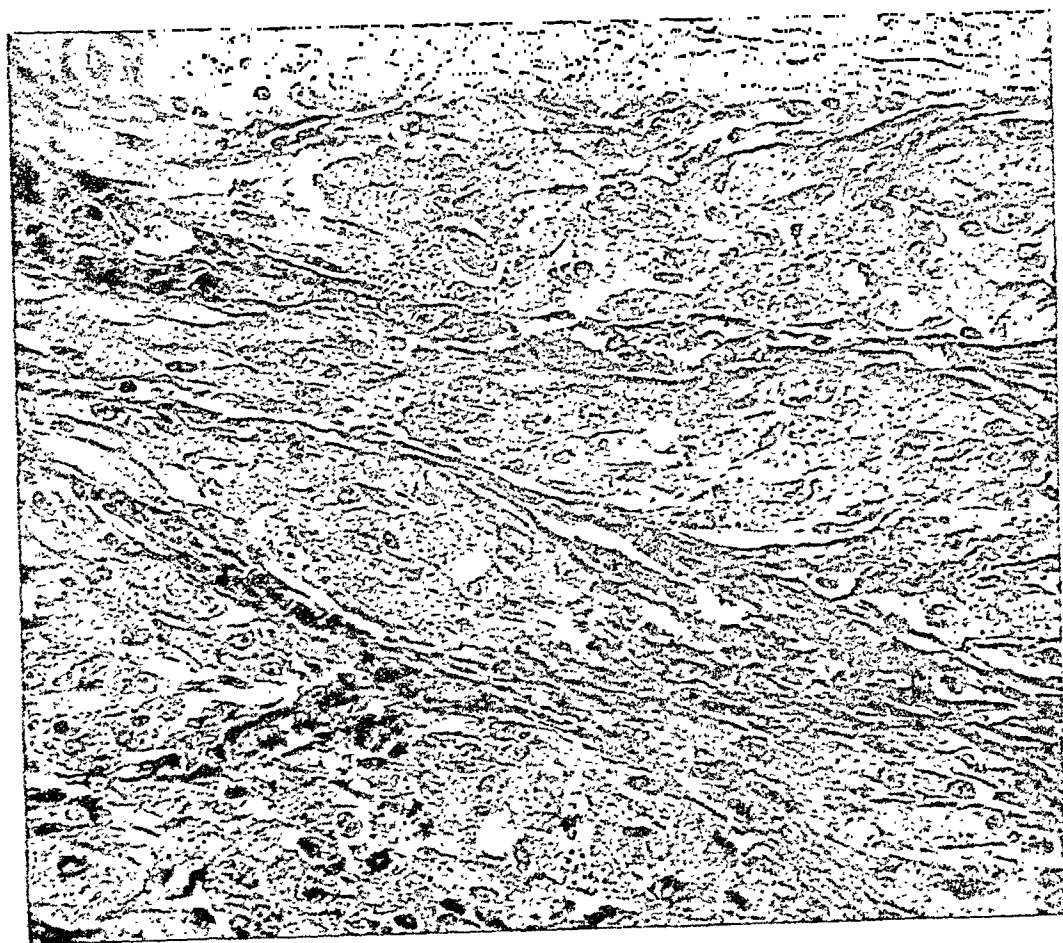
The tumor cells are supported on thin, poorly vascularized and somewhat delicate connective tissue septa which enclose groups of cells giving at times an alveolar appearance to the tumor. With Masson's trichrome stain many delicate connective tissue strands are found to penetrate these groups of cells and to surround individual cells. Frequently, groups of granular cells lie in apposition to each other and fail to show supporting fibrils and under these circumstances present a suggestive syncytial arrangement. Sections stained for fat fail to reveal the presence of lipid of any type and glycogen stains, in our hands, have given consistently negative results. The cytoplasm of these tumor cells is never vacuolated. In none of the present cases was any proliferation of overlying epidermis or mucous membrane noted, although this has been emphasized by Keynes¹⁵ and Klemperer.¹⁶

FIG. 3. DETAILS OF GRANULAR CELLS AND SUPPORTING STROMA FROM GRANULAR CELL MYOBLASTOMA OF BREAST, CASE 1. HEMATOXYLIN AND EOSIN. X580.

FIG. 4. GRANULAR CELL MYOBLASTOMA OF SKIN OF THIGH, CASE 5. The granular cells are enclosed in coarse connective tissue envelopes giving the tumor a somewhat alveolar appearance. Masson trichrome. X335.



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DISCUSSION

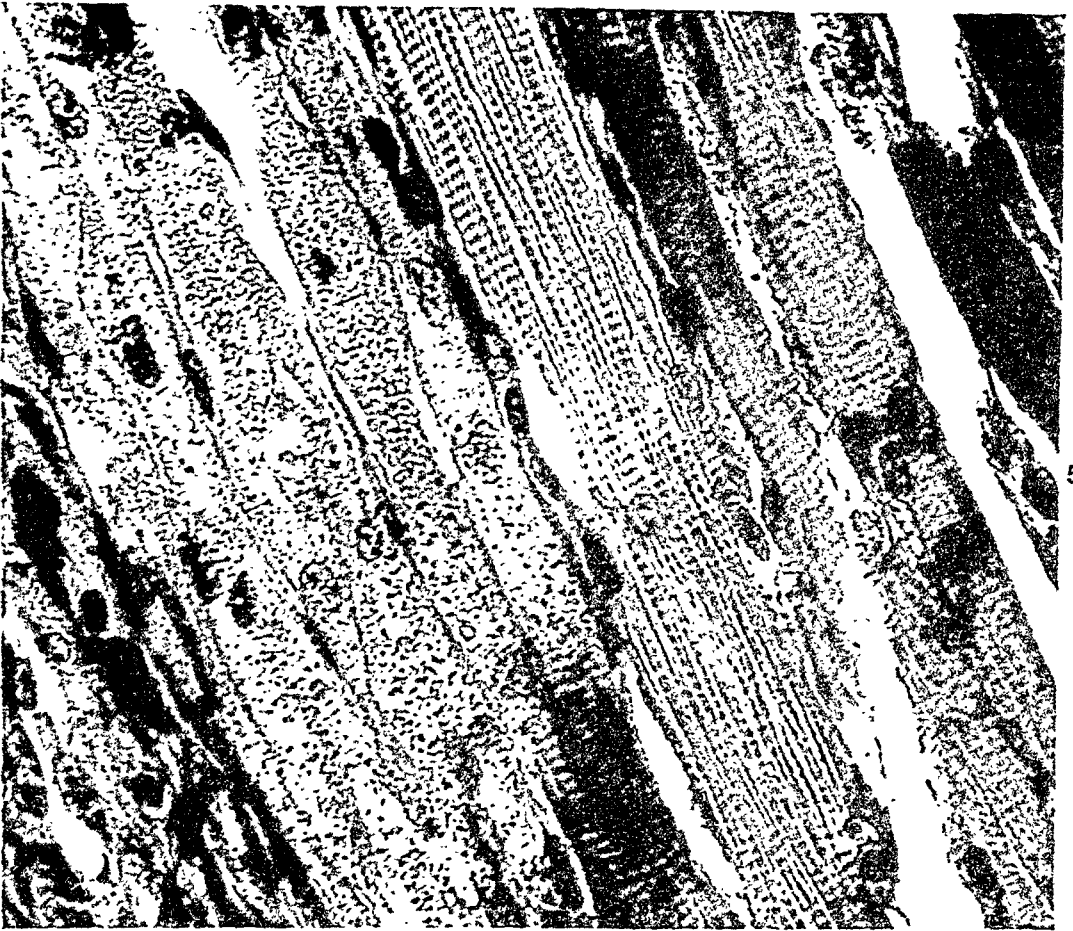
Opinions as to the derivation and nature of these tumors differ. The tumors which Abrikossoff¹ reported in his original communication all arose from situations in which striated muscle is ordinarily present. Indeed, a variable degree of intimate admixture of striated muscle fibers and granular tumor cells is a constant feature in all granular cell tumors when they arise in these situations. Under these circumstances there frequently appears to be, on first glance, transitions from identifiable cross striated muscle fibers to granular cells, and some of the granular cells seem to be continuous with muscle fibers. In the great majority of such findings, careful focusing under oil immersion will show that this apparent transition is not real and that actually a tangentially cut muscle fiber overlays a granular tumor cell. Only fortuitously can undoubted transition from striated muscle fibers to granular cells be identified. Since individual skeletal muscle fibers do not always lie in the same plane and since occasional anastomoses occur, it can easily be seen how these may assume polyhedral or circular forms in various planes of section. Once the granular character in a fiber is established the cross striations appear to be lost.

When granular cell tumors appear in situations where skeletal muscle is ordinarily not encountered, the problem of origin of these tumors becomes somewhat more complex. Under these circumstances an admixture of granular cells and undoubted skeletal muscle fibers is not seen and the tumor is composed entirely of large circular granular cells. Klinge,¹⁷ therefore, interpreted these tumors as being derived from "myoepithelial" structures, but Abrikossoff,² in his second report, felt that embryologic rests (in the sense of Cohnheim) of myoblasts in various situations could account for sites of origin for myoblastic myomas anywhere in the body. While most investigators accept this concept to explain the development of myoblastic myomas in situations in which striated muscle does not ordinarily occur, Gray and Gruenfeld¹¹ and Gander⁹ were of the opinion that tumors arising in these locations were not histogenetically identical with granular cell tumors arising within skeletal muscle. The striking similarities between the cells of these tumors regardless of their location cannot be ignored and the majority of investigators regard all these granular tumors as of myoblastic origin.

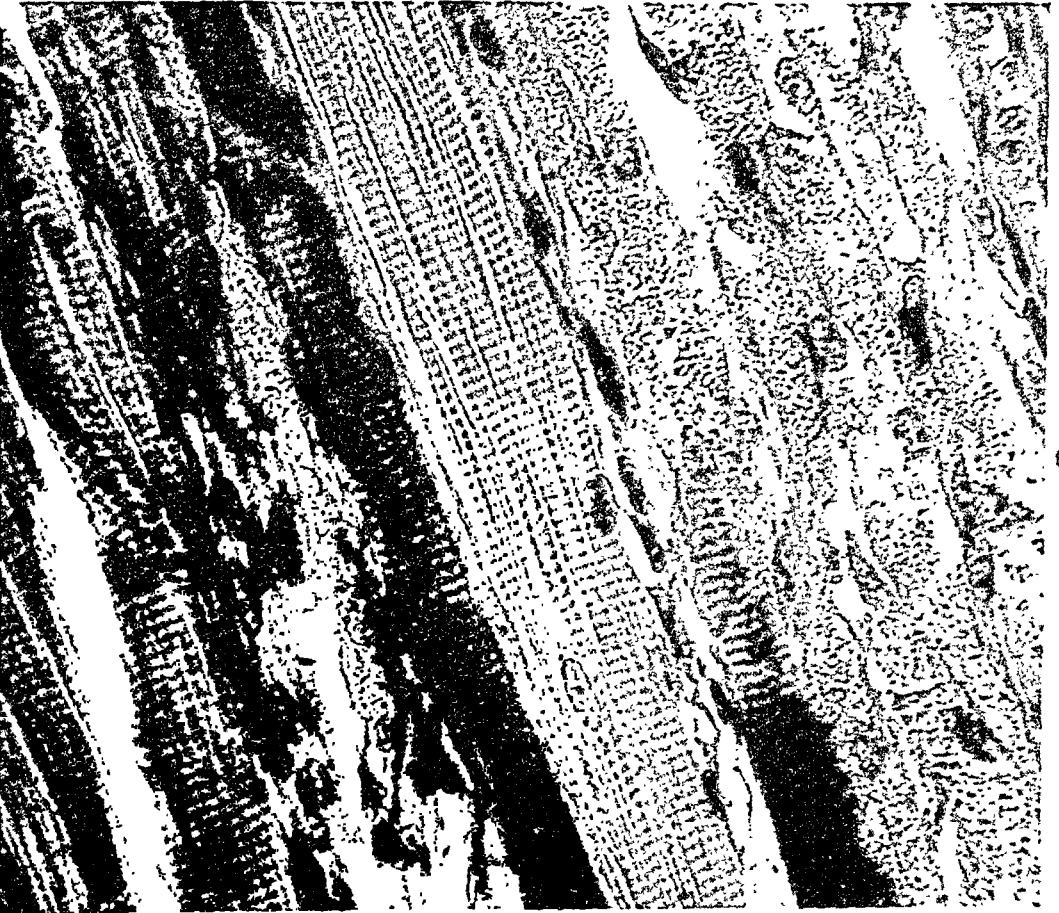
The question as to whether myoblastic myomas simply represent degenerative processes in pre-existing skeletal muscle or are true tumors composed of proliferating myoblasts is debatable. Although Abrikossoff^{1,2} felt that these tumors might be induced by trauma, inflammation or degenerative causes, it is obvious

FIG. 5. PSEUDOTRANSITION OF SKELETAL MUSCLE FIBER TO GRANULAR CELL (CENTER AND JUST BELOW CENTER OF FIELD). In reality this is a skeletal muscle fiber cut tangentially which overlays a granular cell. From Case 2, granular cell myoblastoma of tongue. Masson trichrome. X580.

FIG. 6. GENUINE TRANSITION (TO RIGHT OF CENTER) FROM SKELETAL STRIATED FIBER TO GRANULAR CELL. Under oil immersion this fiber at the point of transition lies in the same plane as the adjacent granular cell. From granular cell myoblastoma of tongue, Case 2. Masson trichrome. X580.



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that he regarded the tumor as due to proliferation of cells which he called myoblasts and, hence, are new growths in the ordinarily accepted sense. The resemblance of these granular cells to so-called myoblasts, as originally described by Godlewski¹⁰ and subsequently by Wolbach,²² forms the basis of the concept that these granular cells are true, newly formed myoblasts. These authors^{10,22} noted a linear arrangement of the cytoplasmic granules which they regarded as precursor forms of myofibrils. Klemperer¹⁶ noted this in one of his cases, but no similar linear arrangement of granules was noted in any of the cases observed by us.

On the other hand, Gray and Gruenfeld¹¹ maintained that these granular cells were simply degeneration products and have been observed as degenerative changes in skeletal muscle following nonspecific ulcerative lesions of the tongue. They cited the work of Zechel,²³ Franz,⁸ and Marcus¹⁸ who made no reference to granular forms of myoblasts in myogenesis. Gander⁹ and Civatte and Ducourtioux⁵ also subscribed to the degenerative nature of these granular cells. It is interesting to note, however, that those authors who regarded these granular cell lesions as degenerative in nature as well as those authors who regarded them as true new growths of proliferating myoblasts have all used the term myoblastoma in some form to designate the lesion. Thus, these tumors have appeared in the literature as myoblastic myoma, myoblastoma, granular cell myoblastoma and rhabdomyome granulo-cellulaire. Since the histogenetic nature of these tumors remains in some doubt, it would appear that the last named term, *i.e.*, rhabdomyoma granulo-cellulaire, would be preferable as it is largely descriptive and avoids the question of origin from primitive myoblasts.

Another important point concerning these granular cell tumors hinges on whether so-called myoblastic myomas are all benign or may show malignant variants. Although the great majority of authors regard these tumors both from a clinical and histologic point of view as benign, Abrikossoff¹ noted a post-operative recurrence in one of his original cases. In 1929, Meyenburg¹⁹ recorded a frankly sarcomatous granular cell tumor and, in 1931, Abrikossoff² classified myoblastic myomas into four types, the last of which he classified as myoblastic sarcoma. At this time, he recorded two cases of myoblastic sarcoma, one each in the esophagus and urinary bladder, in which he found granular tumor cells that he regarded as undoubted granular myoblasts. Horn and Stout¹³ have reported recurrence in granular cell myoblastoma. Howe and Warren¹⁴ stated that 11 per cent of the cases collected and reported by them were malignant and of these, only 3 showed proved metastases. Within the last year there has been recorded a malignant granular cell myoblastoma of the urinary bladder by Ravich, Stout and Ravich,²¹ a malignant granular cell myoblastoma of the gluteal region by Ackerman and Phelps³ and a case of malignant myoblastoma of the skin with metastases to the ovary by Powell.²⁰ The great majority of granular cell myoblastomas are small and their growth is slow or, at times, even stationary. Most of them are both histologically and clinically benign. It is, however, important to recognize that they should be completely removed to avoid recurrences and that malignant variants do occur. In none of the present series of cases has recurrence been observed.

While no malignant granular cell myoblastomas have been reported in the breast, Haagensen and Stout¹² have called attention to the fact that these tumors in the breast may, in the gross, closely simulate carcinoma and that frozen sections must be performed to clearly differentiate these tumors from carcinomas. It is interesting to note that granular cell myoblastomas in the breast and in other situations in the body have been erroneously diagnosed as xanthomas.

SUMMARY

Six additional cases of granular cell myoblastoma (rhabdomyome granulocellulaire) have been reported. These tumors occur most frequently in situations where skeletal muscle normally exists, but may be found in many situations where skeletal muscle normally does not occur. The nature and histogenesis of these tumors is not definitely settled. While the great majority of granular cell myoblastomas are slow growing and probably benign, they should be completely removed to avoid recurrence. Malignant variants of granular cell myoblastomas occur.

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ACUTE LIVER NECROSIS IN FULMINATING INFECTIOUS HEPATITIS*

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During the recent war, our knowledge of the pathology of infectious hepatitis became clarified through the opportunity of studying many patients with the disease, under hospital conditions, and by means of liver biopsies. Excellent articles by Hardy and Feemster³ and by Sodeman⁷ summarize the facts that have accumulated. Dible,² by means of liver biopsies, and Lucké,⁴ in his classic paper on the postmortem findings in 125 fatal cases, showed that the essential lesion was necrosis of the parenchymatous liver cells.

It is known that the liver has a remarkable power of regeneration which is reflected in the fact that the vast majority of the patients suffering from infectious hepatitis make an uneventful recovery with complete resolution of the liver damage, as proved by biopsies. In other cases, fibrosis and nodular regeneration may occur resulting in the picture of cirrhosis or subacute necrosis with multiple nodular hyperplasia. A few cases, however, are seen that present an acute fulminating syndrome with a rapidly fatal outcome. It is the purpose of this paper to present the lesions found in the liver in four such cases seen in the European theater of operations in the autumn of 1944 and the spring of 1945. In Lucké's⁴ series, the earliest death occurred on the tenth day, whereas in this group the longest period of illness was only five days.

Two of the patients received blood and plasma after wounding, and their disease might be classified as homologous serum jaundice, since the time interval between their transfusions and the onset of symptoms agrees with the longer incubation period attributed to homologous serum jaundice. It is not possible to be definite on this point, however, since the illnesses occurred during a period when many cases of straightforward infectious hepatitis were seen. One patient appeared to have so-called arsenotherapy jaundice, while the other seemed to have infectious hepatitis, since there was no history of either inoculations or parenteral therapy of any description. Such a classification, however, would appear to be irrelevant since the pathologic changes seen in each of these "types" is identical and very possibly the same etiologic agent is involved. The recent immunologic experiments of Neeffé *et al.*,⁶ however, suggest that different agents may be concerned in the production of homologous serum jaundice and infectious hepatitis.

REPORT OF CASES

Case 1

Clinical data. A 40 year old officer received, on October 10, 1944, multiple grenade wounds involving the legs, thighs and arms, and a compound fracture of the left tibia. He

* Received for publication, November 19, 1946.

was given plasma transfusions and was evacuated to a base hospital in England, on October 17. Three days later, his wounds were sutured, the fracture explored and set, and 1000 cc. of plasma was given, followed by 1000 cc. of homologous whole blood. His convalescence was quite uneventful until December 17, when he complained of malaise, headache and upper abdominal pain. Three days later, he became mildly icteric, bilirubin appeared in the urine and the icterus index was raised to 25. The following day he was very restless and delirious. The white blood cell count was 27,200 per cu. mm., with polymorphonuclears predominating. The cerebrospinal fluid contained 24 lymphocytes per cu. mm., and Pandy's test was 1 plus. He gradually became comatose and expired the following afternoon, five days after the onset of symptoms.

Autopsy findings. The patient was mildly jaundiced. The liver weighed 910 gm., was small and flaccid with a dusky red smooth surface. The cut surface was friable and had a mottled appearance rather similar to a "nut-meg" liver, due to alternating thin red and yellow bands. No large areas of hemorrhage or necrosis, so commonly seen in acute yellow atrophy, were present. The gallbladder appeared normal and contained a thick green mucoid bile that was expressed with difficulty through the common duct. The common and the hepatic ducts appeared normal. The brain felt heavy but no particular pathologic changes were noted. The spleen was moderately enlarged, soft, mushy and congested. The remaining viscera appeared normal, while the compound fracture of the left tibia showed healing and no infection was present.

Microscopic findings. The normal architecture of the liver was completely lost due to massive necrosis and disintegration of the columns of liver cells (Fig. 1). This process was diffuse throughout the entire liver. In the periportal areas a few minute irregular islands of distorted liver cells remained. These resembled syncytial masses with irregularly placed nuclei due to the rupture of the normal cell boundaries (Fig. 2). The cytoplasm was vacuolated, while many of the nuclei showed karyorrhexis. Surrounding these nubbins of liver cells, and particularly in the portal tracts, was a moderate round cell infiltration consisting of histiocytes, lymphocytes, plasma cells and an occasional polymorphonuclear leukocyte. The bile ducts were prominent but showed no proliferation, nor were their cuboidal cells involved with the necrotic process. No bile thrombi were found. The remainder of the lobules consisted of the sinusoidal framework containing an eosinophilic amorphous material (presumably products of liver cell disintegration) and outlined by the prominent, swollen reticulo-endothelial cells (Kupffer's cells). A few small remnants of liver cells still containing nuclei were distinguished. The majority of these contained a golden brown granular pigment which failed to give a positive test for hemosiderin. A few of the histiocytes had phagocytosed this pigment. Scattered throughout were minimal numbers of lymphocytes, plasma cells and an occasional polymorphonuclear. Many of the portal vein branches showed a mild round cell infiltration involving the media and intima (Fig. 3). The lumens, however, were everywhere patent.

There was a patchy meningitis, most marked in the depths of the cortical sulci, which contained a cellular exudate of histiocytes, lymphocytes and a few polymorphonuclears. The adjacent parenchyma was edematous and granular. Scattered in it were numerous structureless globules which stained uniformly with hematoxylin. In some areas, these were arranged about small blood vessels, but there was no lymphocytic "cuffing" present. A few similar droplets were scattered in the deeper parenchyma, where they were present in much smaller numbers. Similar changes were noted in the medulla where the droplets formed a conspicuous band immediately beneath the surface. No gliosis was seen, but a mild patchy neuronophagia was present.

Case 2

Clinical data. A medical officer suddenly became ill on December 27, 1944, complaining of chills, general malaise and despondency. He had been in excellent health up to this time except for a mild upper respiratory infection and general tiredness, attributed to overwork in handling casualties. Within an hour of the onset of his illness, he was admitted

to the hospital where his depression was found to be a striking feature. He had previously been caring for the patient of Case 1 and declared on admission that he had the same disease, although at this time there was no visible jaundice. His illness quickly became serious, his depression more marked, and tenderness developed beneath the costal margin. On the following day, icterus appeared, bilirubin was positive in the urine and its level was increased in the blood serum. The leukocyte count averaged 17,000 per cu. mm., the majority being polymorphonuclears. Examinations of the cerebrospinal fluid were negative.

Treatment consisted of intravenous glucose saline and methionine, but the patient rapidly deteriorated, became dehydrated, delirious, then comatose and expired on January 31, 1945, five days after the onset of symptoms. No history was obtained that the patient had pricked a finger during operation, or that there was any other obvious method of contamination.

Autopsy findings. The liver was shrunken, flabby and dusky red. The surface was smooth and covered with a wrinkled capsule. The cut surface was friable and presented a "cooked" mottled appearance, thin-deep red areas alternating with similar mustard colored ones. The spleen was soft and toxic in appearance. The remaining viscera did not reveal any pathologic changes. The brain was not examined.

Microscopic findings. The changes in the liver were practically identical with those described in the above case, except that the process appeared even more marked. There was complete disintegration of all liver cells, the few small remaining fragments bearing no resemblance to normal hepatic cells. These contained a pigment similar to that seen in Case 1. In addition to the diffuse necrotic process, there was a marked fatty metamorphosis, large globules of fat forming a lace-work in many areas. The inflammatory cell reaction was also more marked, particularly in the portal areas where there was a heavy infiltration of round cells with a few polymorphonuclears (Fig. 4). The involvement of the branches of the portal vein was also accentuated. The bile ducts appeared normal and did not show any proliferative activity.

Case 3

Clinical data. A 22 year old soldier presented himself with symptoms of general malaise and chills. He had been in good health previously. About six weeks before, he had completed a course of arsenotherapy for lues which now appeared under control. He was treated at his regimental aid post for twenty-four hours when he became drowsy. A meningitis was suspected and he was admitted to a forward hospital. The cerebrospinal fluid was clear, but there were 35 cells per cu. mm. There was a polymorphonuclear leukocytosis of 18,000 per cu. mm. and a slight icteric tinge to the skin and sclerae. He rapidly deteriorated, became comatose and died five days after the onset of symptoms.

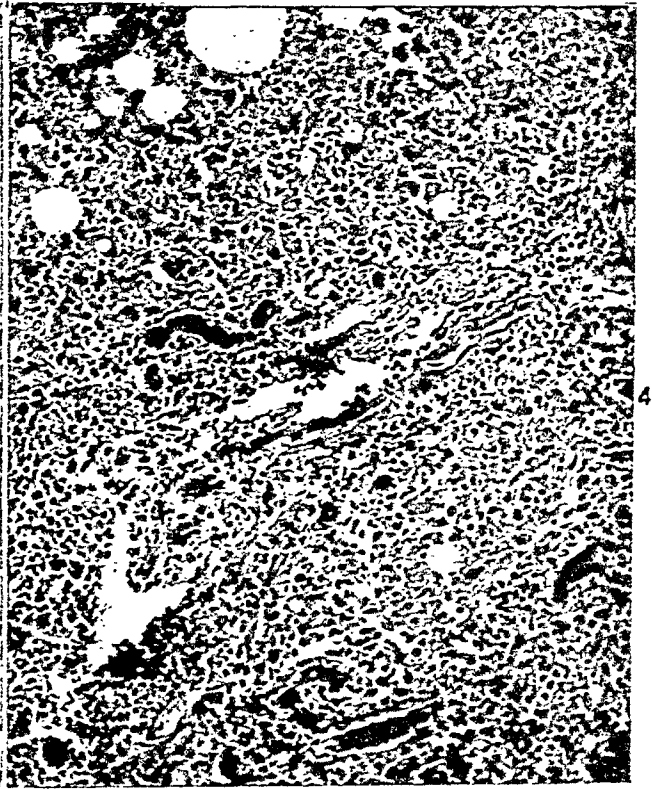
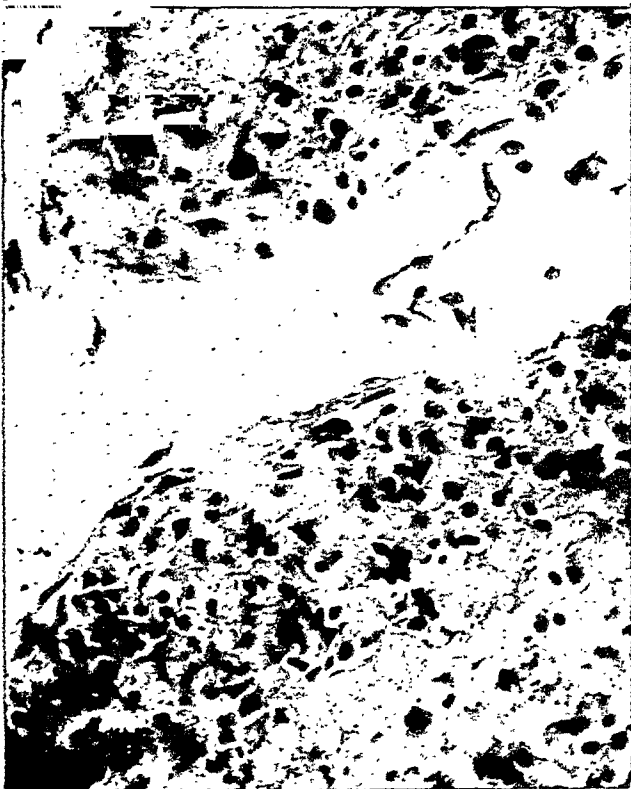
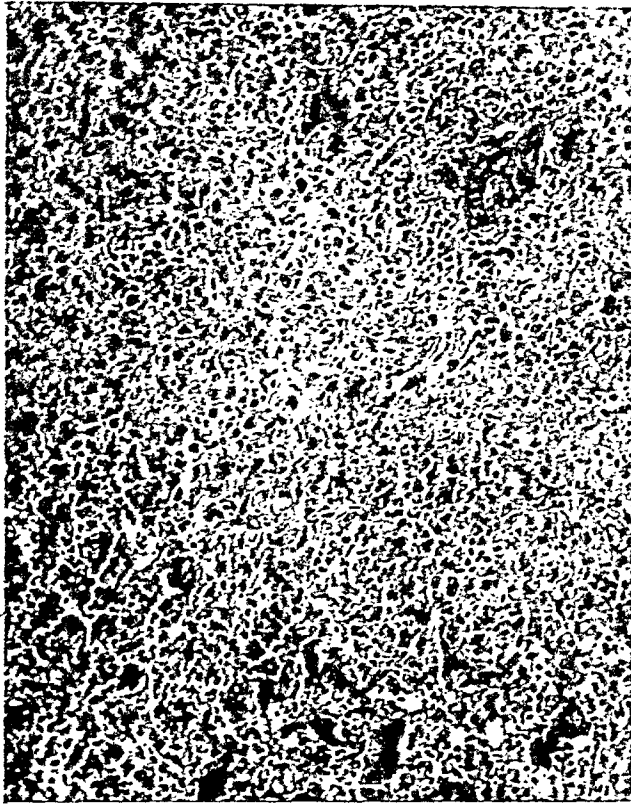
Autopsy findings. The liver, spleen, brain and kidneys were removed and sent to the laboratory of a base hospital for examination. Gross examination of the fixed liver revealed a similar picture to that seen in Cases 1 and 2. Although the liver felt firm because of fixation, the tissue of the cut surfaces was friable. The brain was edematous and its

FIG. 1. Case 1. Low power view showing marked disintegration of columns of liver cells and round cell infiltration resulting in complete loss of architecture. At the edges can be seen a few large "nubbins" of disintegrating liver cells. Hematoxylin and eosin. X97.

FIG. 2. Case 1. High power view of groups of liver cells in periportal area similar to those at edge of Fig. 1. Note the loss of cell boundaries, the marked vacuolation of cytoplasm and pyknotic nuclei. They are surrounded by lymphocytes and histiocytes. Hematoxylin and eosin.

FIG. 3. Case 1. Inflammatory cell infiltration of the wall of branch of portal vein. Hematoxylin and eosin.

FIG. 4. Case 2. Low power view in region of portal triad showing fatty change, necrosis and disintegration of liver cells and heavy lymphocytic infiltration. Hematoxylin and eosin.



convolutions flattened. No gross exudate was noted on the surface of the brain. The spleen was slightly enlarged and toxic in appearance.

Microscopic findings. Blocks from all lobes again revealed the same pathologic features noted above. In some areas, there remained small nodules of completely necrotic liver cells with a smudged appearance and with masses of greenish golden pigment (Fig. 5). Elsewhere, there was marked fragmentation and disappearance of the cells, leaving an empty framework in which were scattered small, irregular, deeply pigmented, necrotic-appearing liver cell fragments (Fig. 6). The Kupffer's cells were very prominent, standing out sharply against the empty framework. A round cell infiltration, similar to that previously illustrated, was present, particularly in the portal tracts where the portal veins were again involved. The bile ducts were normal. Silver stained sections revealed an intact reticulum still having the normal lobular pattern (Fig. 7). Histologic sections through the midbrain revealed a superficial softening in which appeared structureless, hematoxylin-staining droplets similar to those mentioned in Case 1. They were not as prominent, however, and there was no meningeal involvement.

Case 4

Clinical data. A soldier received a penetrating chest wound with hemothorax which subsequently clotted and became infected. He received plasma transfusions along the line of evacuation and whole blood at the base hospital. Under penicillin therapy and appropriate surgical procedures, he made a good recovery with reexpansion of the lung. He was completely convalescent and doing light ward duties when he developed chills with anorexia. On the second day, he became jaundiced and delirious, subsequently lapsing into coma and expiring four days after the onset of this illness.

Autopsy findings. The liver was shrunken and soft with dusky red smooth surface. The cut surface was friable and had a mottled appearance due to irregular alternating areas of soft red and yellow tissue. The bile ducts were normal. The brain appeared normal on gross examination. The lungs revealed fibrous adhesions between the parietal and visceral pleurae, but no exudate was present. Fibrosis and scarring surrounded the wounds from which the shrapnel fragments had been removed. The spleen was slightly enlarged, soft and mushy.

Microscopic findings. There was diffuse necrosis of the liver cells. In most areas, however, the outlines of the cells remained. In many, the nuclei had completely disappeared, while in others, karyorrhexis and pyknosis were present (Fig. 8). Many of these cells contained a granular yellow pigment. The endothelial cells were prominent and there was mild round cell infiltration of the portal areas with involvement of the portal veins. Sections of the brain were normal.

DISCUSSION

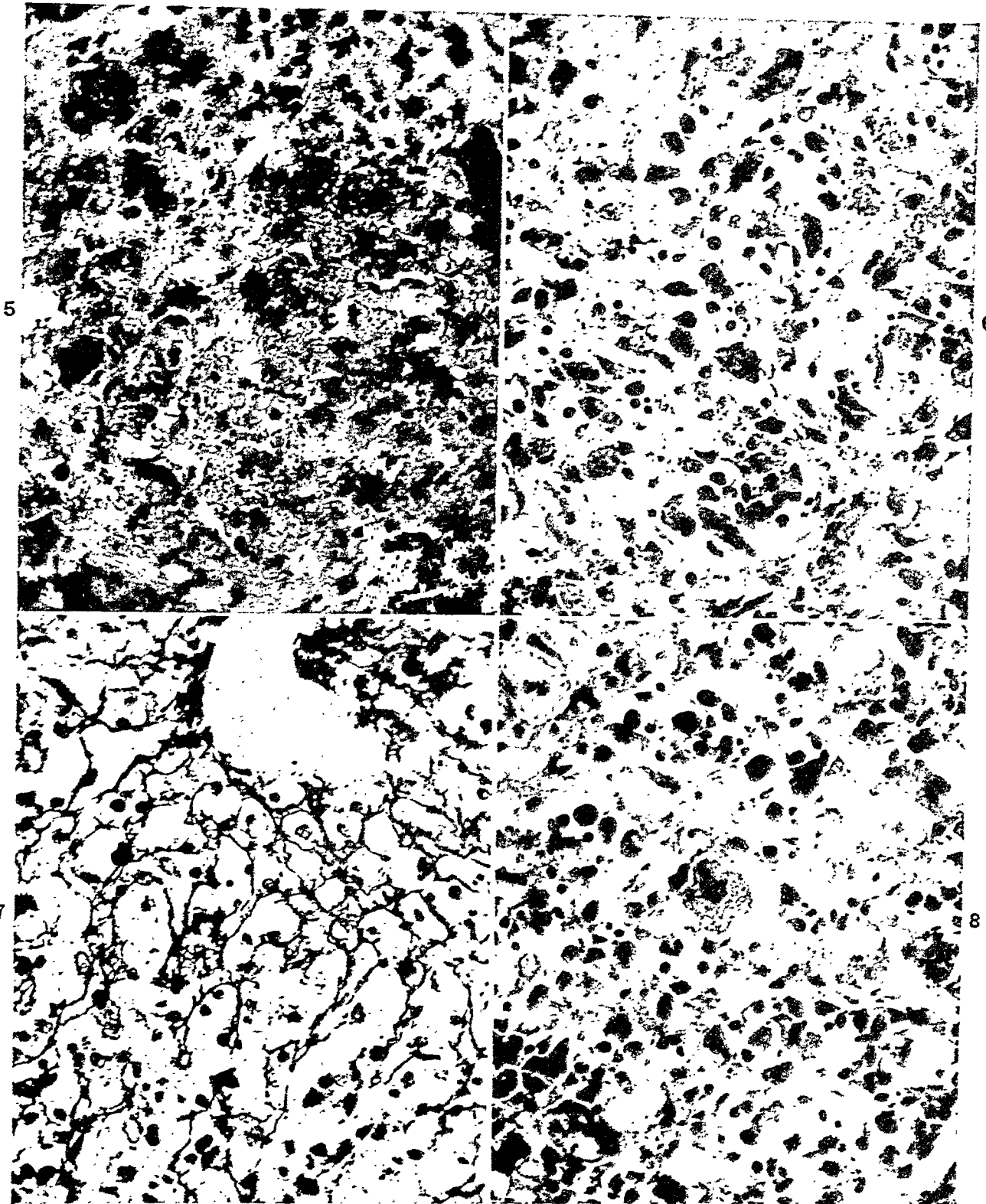
The pathologic findings in the liver were identical in all four cases. In summary, they consisted of a widespread necrosis, fragmentation and disappearance of the liver cells. The portions of cells remaining, which were about one-third

FIG. 5. Case 3. Small nodule of completely necrotic liver cells with smudged appearance containing granular pigment. Only a few such nodules were present in each section. Hematoxylin and eosin.

FIG. 6. Case 3. Note the marked destruction of liver cells leaving relatively empty framework. The few fragments of liver cells contain a granular pigment. Hematoxylin and eosin.

FIG. 7. Case 3. Silver stained section of liver showing the intact reticulum radiating outward from a central vein in the upper portion of the field. Wilder's reticulum stain.

FIG. 8. Case 4. The fragments of necrotic liver cells with pyknotic nuclei can be seen scattered in the lobular framework together with lymphocytic infiltration and histiocytes. Hematoxylin and eosin.



the size of normal cells, contained, in many instances, a golden brown pigment similar to that described by Lucké⁴ as being a lipochrome, the product of cellular disintegration. In some of the slides, this had also been phagocytosed by the histiocytes. There was a varying degree of round cell infiltration, most marked in the portal areas and involving the walls of the branches of the portal vein. The Kupffer's cells were swollen and were exceptionally prominent, standing out sharply in the relatively empty framework. The reticulum in the one case examined was quite intact.

These changes were practically identical with those already described by Dible² and Lucké⁴ in cases of infectious hepatitis, homologous serum jaundice and arsenotherapy jaundice, with the exception that they were much more widespread. In the many blocks examined from each of the cases, not one completely normal liver cell was seen and there was no evidence of any bile duct proliferation or fibrous tissue replacement.

The selectivity of the agent involved was striking. With uncanny regularity, it affected the liver parenchymal cells, sparing the framework and biliary epithelium. The rapidity of its action was frightening, when one considers a disintegration of practically all liver cells within a period of four or five days. This was also reflected in the extremely rapid wasting of the organ. It was estimated in Case 1 that there had been a loss in weight of liver of approximately 600 gm. in the five days of illness.

Clinically, the most striking feature of the patients was apathy, which was prominent from the beginning and was quickly followed by delirium and coma. To what extent this physical state is explained by the concomitant finding of a peculiar meningo-encephalitis is difficult to assess. In two of the cases, the involvement of the central nervous system was prominent; in one, the brain was not examined; while in the fourth case, the available slides failed to show any changes in the brain, although this patient presented the same clinical picture. Lucké,⁴ in his series, found an associated mild meningo-encephalitis in 15 per cent of cases.

Since this paper was prepared, Lucké and Mallory⁵ have published an excellent detailed article on the fulminant form of epidemic hepatitis, which fully confirms the pathologic findings described here. It is interesting that in their larger series, they did not find the same degree of involvement of the central nervous system that was seen in Cases 1 and 3, although they stated that a mild nonspecific encephalopathy was nearly always observed.

SUMMARY

Four fatal cases of fulminating liver necrosis in infectious hepatitis, homologous serum jaundice and arsenotherapy jaundice have been presented. The length of illness from onset of symptoms to death, was four or five days.

The essential lesion was an extremely rapid diffuse necrosis and disintegration of the parenchymal liver cells. No normal cells were found in the many blocks examined.

There was an associated meningo-encephalitis in two of the cases.

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AIR EMBOLISM OCCURRING DURING MASTECTOMY: REPORT OF A FATAL CASE*

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Air embolism is discussed in the majority of standard textbooks on pathology and surgery without reference to the frequency of occurrence. The impression is gained from such texts that it is a common complication following surgical procedures about the neck and shoulders.

After a fatal case of air embolism complicating a mastectomy, the only such occurrence during 451 mastectomies performed at the Geisinger Memorial Hospital, we searched the literature for similar instances. Cases of air embolism following surgical procedures about the shoulders and neck have been very rarely reported, and to our knowledge no such accident complicating a mastectomy has been reported within the past 30 years.

Furthermore, from personal communications received from several pathologists associated with active departments of pathologic anatomy, air embolism as a cause of death in their experience has seldom been encountered. A few of the aforementioned pathologists have observed this condition as a complication of artificially induced pneumothorax and/or following vaginal insufflation. None reports having observed air embolism resulting from other surgical procedures.

For these reasons it seemed worth while to record such an occurrence.

REPORT OF CASE

A 37 year old female noticed a small hard mass in her left breast approximately five months prior to being examined at this hospital. Physical examination was essentially negative except for the presence of a hard, freely movable mass, approximately 6 cm. in diameter, in the outer upper quadrant of the left breast. The preoperative medication used was sodium pentobarbital gr. $1\frac{1}{2}$ one hour prior to operation and morphine sulphate gr. $1/6$ plus atrophine sulphate gr. $1/150$ on call to the operating room. The anesthetic agents employed were cyclopropane, nitrous oxide, oxygen and ether. Tissue was removed for biopsy and a rapid frozen section revealed adenocarcinoma. A radical mastectomy was then begun with the patient in good condition. Suddenly, after removal of the breast, but with the muscles and axillary fat still intact, a loud sucking noise was heard, followed in two or three seconds by a similar sound. The sucking noise began simultaneously with the severing of a branch of the axillary vein. The patient's color immediately became ashen, the respirations shallow and irregular, the blood pressure fell from 120/70 to 70/0 and the pulse was poor in quality and its rate 60 per minute. The bag was emptied of anesthetic agents and 100 per cent oxygen was administered by manual compression of the bag with an

* Received for publication, December 20, 1946.

endotracheal tube in place, but the patient's color did not improve. The blood pressure continued to fall and the pulse became progressively weaker. Papaverine hydrochloride gr. 1/2 was then administered intravenously. Approximately ten minutes after the onset, the pulse was imperceptible and the blood pressure could not be recorded. Respiratory efforts persisted for several more minutes and then ceased. Artificial respiration was continued with the gas machine for one-half hour after which time the patient was pronounced dead.

Autopsy Findings

There was a recent left mastectomy incision, 21 cm. long. When the pericardial cavity was opened, the right auricle was found to be markedly distended with gas. Prior to opening the heart, the pericardial sac was filled with water so that the organ was completely immersed. The heart was opened *in situ*. When incised, there escaped from the right auricle approximately 60 cc. of gas and only a small amount of red frothy fluid. Numerous small gas bubbles were observed in the coronary arteries. There were two interauricular septal openings, one 1.4 cm., the other 0.3 cm. in diameter. Numerous small gas bubbles were present in the mesenteric and internal mammary arteries.

The diagnoses were recent left simple mastectomy, venous and arterial types of air embolism and interauricular septal defects.

DISCUSSION

In order for air embolism to occur during a surgical procedure, it is necessary for a distended vein having a negative pressure to be severed, leaving the proximal orifice patent.

The pressure in the right auricle has been shown to vary from -35 to -80 mm. of water. This variation in the venous pressure is due to the changes in intrathoracic pressure. During the inspiratory phase of respiration the negative intrathoracic pressure is increased which, in turn, increases the negative venous pressure in the right auricle, thereby aiding in sucking blood into the chest. The increased negative pressure existent in the right auricle is transmitted with a decreasing gradient through the peripheral veins until the point is reached where the pressure in the veins becomes zero and then positive. Therefore, the point of zero pressure in the venous system would necessarily vary in distance from the right auricle, depending primarily upon the changes in the intrathoracic pressure. The point of zero pressure is more distal to the heart during inspiration than during expiration. Because two distinct sucking noises were heard in this patient, one may assume that the vein sectioned had a negative pressure only during inspiration and a zero or positive pressure during the expiratory phase of respiration.

The volume of air in the circulatory system which is necessary to produce death in man is not known. The rate of entrance of the air greatly influences the quantity that is lethal.^{1, 2, 3} The more rapid the entrance of air into the circulatory system, the smaller the volume required to produce fatality and, conversely, the slower the entrance of air, the greater the volume required.

Two types of air embolism have been described, the venous or pulmonary and the arterial or systemic. During surgical procedures about the neck or shoulders, barring anatomic anomalies, it is the venous type of air embolism which may occur. When this happens, if the volume of air is not too great, the lungs will filter the air from the blood stream before it reaches the arterial circulation, and thus prevent damage to the brain. In this patient, both types of air embolism were found. This occurrence is readily explained by the presence of the two interauricular septal defects, and by the development of a pressure in the right auricle greater than that in the left auricle. The air entered the axillary vein, passed to the right side of the heart and to the pulmonary arterial bed. It is believed that the pressure in the pulmonary arterial bed was quickly raised and that this increased pressure was transmitted back to the right side of the heart. Air and blood were then forced from the right auricle through the patent interauricular septal defects into the left auricle and thence into the arterial circulation.

SUMMARY

An instance is reported of fatal air embolism, which occurred during a mastectomy in a 37 year old white woman. At autopsy, both arterial and venous types of air embolism were found.

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FATAL CEREBRAL COCCIDIOIDOMYCOSIS

REPORT OF CASE*

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A fatal case of coccidioidomycosis affecting the cerebrum is presented. This disease is encountered in two forms, primary coccidioidomycosis which is a benign form, and progressive coccidioidomycosis which occurs about once in 500 cases. This infection is endemic in South Central California, especially in the San Joaquin Valley, and in the southern half of Arizona, western Texas and in New Mexico. The disease now assumes increased importance because troops have been exposed in endemic areas. Thirteen cases of cerebral coccidioidomycosis have been reported by the Army Institute of Pathology.³

The clinical course of this case is similar in many respects to the case reported by Hans G. Schlumberger.³ There were no cutaneous lesions and the terminal phase of coma was of relatively short duration.

This condition is caused by infection with chlamydospores of *Coccidioides immitis*. Man acquires the infection from an exogenous source, most likely from soil contaminated with fungus. Strains of the fungus pathogenic to animals have been cultivated from the soil in endemic areas, and it is thought that the fungus is breathed into the lungs with dust or introduced into the skin following injury. The fungus has not been found growing on any vegetative material. It is possible that the organism is primarily an animal pathogen and that contamination of the soil is maintained by infected rodents.²

REPORT OF CASE

Clinical data. A male, aged 24, was admitted to the Veterans Service of the Philadelphia Naval Hospital on October 19, 1944. He had been in reasonably good health until four months previously when he developed headache, fever and cough. These symptoms persisted and became progressive. He also complained of night sweats, stiffness of the neck, occasional blurred vision and a loss of twenty pounds in weight. He was admitted with the tentative diagnosis of pulmonary tuberculosis, but careful study failed to confirm this diagnosis. His temperature was 99 F., pulse rate 90, respiratory rate 20, blood pressure 95/70. General physical examination was negative except for poor development, moderate emaciation and poor expansion of the chest. Neurologic examination was negative except for blurred vision and stiffness of the neck. X-ray films of the skull and chest were negative. The laboratory findings were as follows: repeated examinations of sputum, negative for acid-fast bacilli; culture of cerebrospinal fluid, no growth at end of seventy-two hours; blood and spinal fluid Kahn tests, negative; hemoglobin, 12 gm.; erythrocytes, 4,230,000; leukocytes, 6950; segmented leukocytes, 69 per cent; lymphocytes, 26 per cent; eosinophils, 5 per cent; sedimentation rate, 26 mm.; urinalysis, negative; spinal puncture, pressure 440 to 510 mm. of water; chlorides, 584; leukocytes, 280; lymphocytes, 80; protein, 100 mg. Repeated spinal punctures showed from 280 to 468 white blood cells. After penicillin was administered, the number of leukocytes in the cerebrospinal fluid was two on each of two occasions. The protein level remained high, ranging from 100 to 200 mg.

* Paper presented before the Philadelphia Neurological Society, May 24, 1946. Received for publication, November 18, 1946.

The patient's complaints persisted. He was cooperative, but at all times appeared acutely distressed. Each spinal puncture afforded the patient relief from his headache for a short period. The neurologic signs gradually increased in severity. The neck became increasingly tender and stiff and could not be flexed toward the chest. The deep reflexes were diminished. Examination of the fundi revealed a bilateral papilledema, blurring of the discs and several retinal hemorrhages about the discs. Seven weeks after admission, he developed a paralysis of the left abducens nerve.

Electroencephalography showed 4 and 6 waves per second and 8 waves per second throughout both hemispheres. Treatment was supportive and symptomatic. Because of

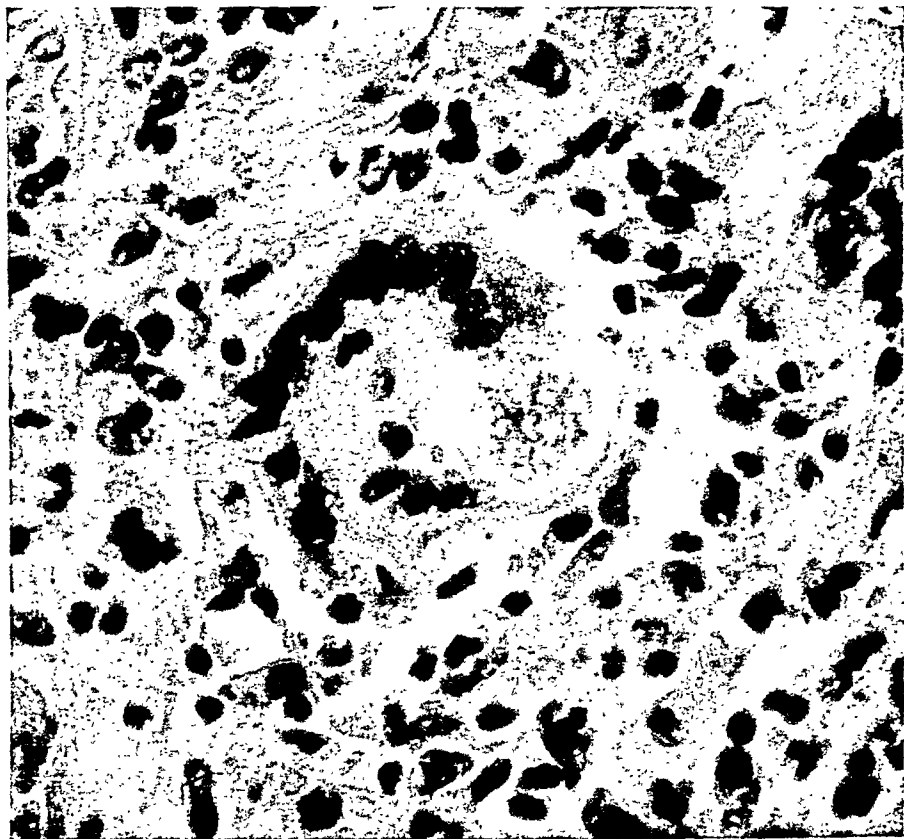


FIG. 1. GRANULOMATOUS REACTION WITH LANGHANS TYPE OF GIANT CELL CONTAINING SPHERULE OF COCCIDIUM AND SHOWING ENDOSPORE FORMATION. $\times 644$.

a history of lues, he was given iodides, bismuth and penicillin. The patient became comatose in the final week of his disease and expired on January 25, 1945, seven months after the onset of his illness.

Pathologic Findings

Gross findings in the brain and spinal cord were as follows. The ventricular fluid was under pressure, but was clear and watery in appearance. The cerebrospinal fluid was increased in amount. The convolutions of the brain were somewhat flattened and the sulci decreased in size. There was, however, no pressure cone over the cerebellum. Over the pons and the lower part of the cerebellum and medulla, there was a pale yellow fibrinous exudate matting these structures together. The upper part of the cervical cord was softer than normal and the normal markings were indistinct. On opening the dura, a pale yellow

fibrinous exudate was present over the sixth, seventh and eighth thoracic segments of the spinal cord, but the spinal fluid appeared to be clear. The brain was pale and the temporal lobe was soft. The arteries were normal. There was an inflammatory reaction in the cisterna ambiens of the subarachnoid space completely occluding the space in that area. In the subarachnoid space of the right antero-lateral aspect of the medulla, there was a firm nodule 1 cm. in its greatest diameter which showed no parenchymal encroachment. The ventricles, including the fourth ventricle, were moderately enlarged.

Microscopic findings. There were unusual lesions of the pia arachnoid of the brain. These showed a chronic granulomatous inflammatory exudate. The most conspicuous

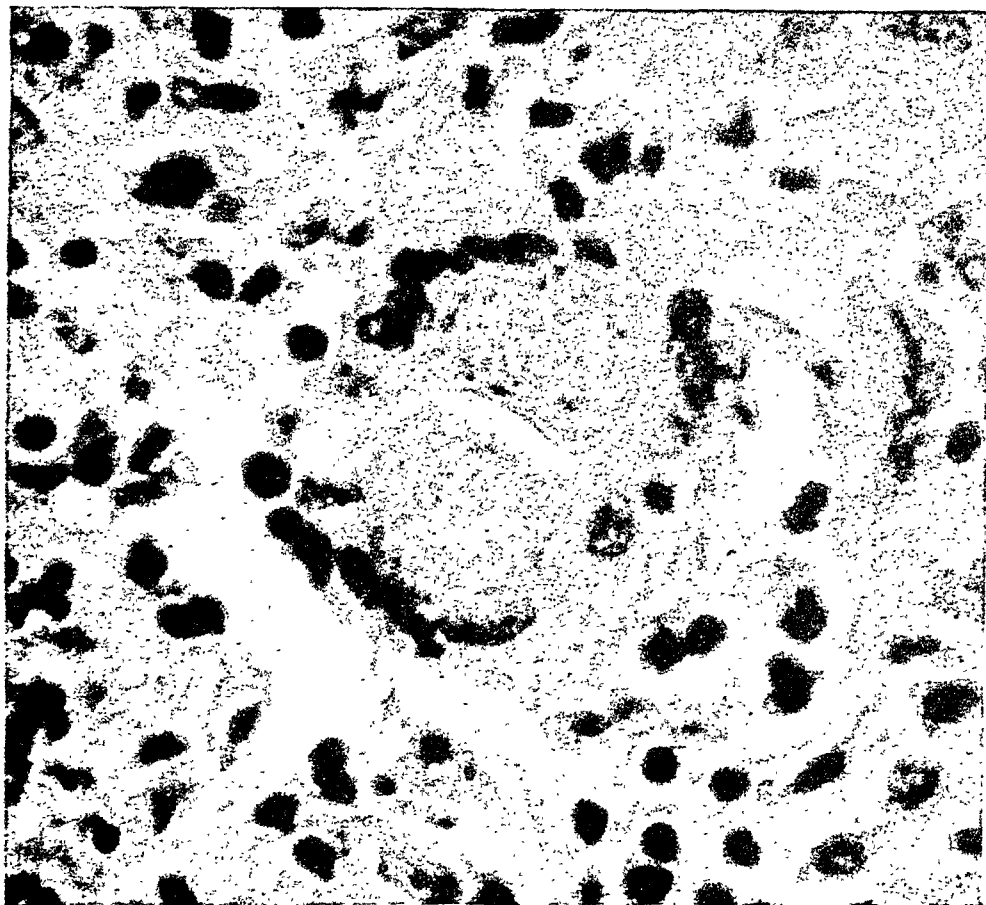


FIG. 2. GRANULOMATOUS REACTION SHOWING SPHERULES OF COCCIDIUM. DOUBLE CONTURED CYST WALL IS WELL VISUALIZED IN THE UPPER SPHERE. $\times 1012$.

cell was the plasma cell, but there were also numerous lymphocytes, polymorphonuclear leukocytes and mononuclear phagocytes. Occasional eosinophils were also seen. Scattered irregularly through this exudate were multinucleated giant cells of the Langhans' type. Although there were foci of necrosis, there were no definite tubercles. The exciting agent of this inflammatory reaction was a fungus characterized by large and small circular cells having thick refractile capsules. Many of these were contained within the Langhans' cells. Reproduction did not appear to be taking place by budding, but by division within the refractile capsule of some of these cells. There was a terminal lobular pneumonia in which none of the organisms could be demonstrated. There was an acute passive congestion of the lungs and kidneys and the regional lymph nodes of the mediastinum. So far as could be determined, there was no primary focus in the lungs or lymph nodes.

COMMENT

Although the cultural reactions of this organism are unknown in this case, the morphologic characteristics were more suggestive of *coccidioides* than of *torula*. The patient's family was uncooperative and his military record could not be obtained. It is not known whether the patient had been in an endemic area of coccidioidomycosis or in contact with a patient suffering from the disease. As far as could be determined, the patient had no focus of infection other than that described.

SUMMARY

A case is reported of cerebral coccidioidomycosis. During life the patient was thought to be suffering from tuberculosis or syphilitic meningitis.

Acknowledgment is made to Dr. Vincent M. Diodati of the Veteran's Bureau, Commanders Luther I. Fisher and Robert Norris of the Philadelphia Naval Hospital, and to Dr. Helena Riggs, neuropathologist to the Philadelphia General Hospital, for their aid in securing histologic illustrations.

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INFECTION WITH *ISOSPORA HOMINIS*

REPORT OF CASE*

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Human infection with *Isospora hominis* is neither common nor serious. The occasional appearance of this little known parasite in the stools of sojourners in the tropics, however, makes the continued reporting of cases desirable.

A description of this parasite is contained in the textbooks of Todd and Sanford⁷ and of Stitt, Clough and Clough.⁶ Magath,⁵ in 1935, summarized approximately 200 human cases reported to date and added a case of his own. Most of the cases were found during World War I among British soldiers in the eastern Mediterranean region. Other cases were reported from China, India, Dutch East Indies, Philippines, Hawaii, eastern coast of South America and from the



FIG. 1. PHOTOMICROGRAPH OF OOCYST OF *Isospora hominis* ISOLATED FROM THE STOOL OF THIS PATIENT

United States (mostly from soldiers serving overseas). The literature was later completely reviewed by Herrlich and Liebmann,² in 1943, who added cases of their own and discussed the morphology and life cycle of the parasites. Further discussion and case reports have been made by Beltran and Larenas,¹ in 1944, Kiskaddon and Renshaw,⁴ in 1945, and Humphrey,³ in 1946.

REPORT OF CASE

The patient, a 26 year old white soldier, a native of Colorado, developed frequent, loose, bloody stools and some rectal pain in mid-November 1945, while stationed in Leyte, Philippine Islands. Antidiarrheal mixtures were given without relief. His appetite was very poor. Although the stools became more formed, diarrhea and abdominal cramps persisted on the boat trip home. A stool was reported negative for *E. histolytica*. The pa-

* Received for publication, January 23, 1947.

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tient had spent two years in the South Pacific, the first two months in Guadalcanal, the next fifteen months in Bougainville, and the last months in Leyte, Philippine Islands. The past history was otherwise noncontributory.

Physical examination upon admission to Fort Logan Station Hospital, Colorado, revealed a well developed, somewhat poorly nourished man, who was not acutely ill, and who had a nasopharyngitis and tenderness to palpation below the umbilicus. There was some excoriation about the anus. Blood pressure was 160/90, pulse rate 96 and temperature 98.8 F. Proctoscopic examination by Captain Donald, of the hospital staff, showed many small punched-out ulcers with grayish membranes and freely bleeding bases, on removal of the end of the instrument. The appearance of these ulcers was consistent with those produced by *E. histolytica*. On two successive days, stools were positive for trophozoites of *E. histolytica*, as well as for occult blood. As an incidental finding, oocysts of *Isospora hominis* were found by zinc sulphate flotation concentration in the stool on three different occasions within a few days. The cysts were few in number and consisted of single and double sporocysts. Studies of urine and blood were not remarkable.

Under treatment with emetine hydrochloride and carbarsone, the patient's stools quite promptly became negative for *E. histolytica*, and the cysts of *Isospora hominis* also promptly vanished. Examinations of the stool repeated two months and four months after the initial positive stool were also negative for *Isospora hominis*.

SUMMARY

An infection with *Isospora hominis* superimposed on amebic dysentery in a soldier recently returned from the Philippine Islands is reported. The *Isospora* promptly disappeared during treatment for amebic dysentery and the stools were still negative four months later.

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EDITORIAL

THE CLINICAL PATHOLOGIST AND THE DIAGNOSIS OF TUBERCULOSIS

In 1943, Harold J. Harris (Bull. N. Y. Acad. Med., 19: 631-655, 1943) indicated the importance of the four recognized laboratory methods in the differential diagnosis of a certain disease. He stressed the importance of the laboratory in specific differential diagnosis and, by way of illustration, he told of the chagrin of the superintendent of one of the best sanatoriums at the reply made to him by a patient. The superintendent had just completed the chest examination of a young woman at a follow-up clinic. "My dear girl," he said, "you do not have tuberculosis. Whoever made such a diagnosis?" The patient answered, "You did, doctor. I spent six months in the sanatorium under your care." He had forgotten that he, himself, had made a clinical diagnosis of tuberculosis and had confined her to bed in spite of negative sputums and only suspicious radiographic findings. Elaborating on this, Hilleboe, Chief of the Tuberculosis Control Division of the U. S. Public Health Service, noted (Pub. Health Reports, 61: 1295-1297, 1946) that no diagnosis of pulmonary tuberculosis should be made on the basis of incomplete evidence. Suspicious findings in the film must be corroborated by a positive tuberculin test and by positive bacillary findings. Treatment should be delayed and judiciously deliberated until all the facts are in and all the evidence evaluated. If such a practice is followed universally, tuberculologists will gain considerably in accuracy and in skill of diagnosis, and limited hospital resources will be conserved. Most important of all, the person suspected of having tuberculosis will be assured thorough and scientific diagnosis and treatment. Judgments based on positive and complete evidence will give a final verdict that protects the individual and the public health. It appears from all this that specific diagnostic data in tuberculosis must be obtained by the clinical pathologist. With such a realization, the clinical pathologist is requested to fulfill the practical essentials for these diagnoses.

There are three specific diagnostic tests (Tuberculology, 7: 108-114, 1945) available at present to disclose tuberculosis. They are of proved merit, simple performance and of inclusive (but not exclusive) value, unless they are not evaluated within scientific limitations. The simplest and oldest of these, the microscopic examination of the stained smear for the presence of acid-fast bacilli, implies a presumptive finding which is limited until verification of the existence of a tuberculous lesion. The test should not be encumbered by overindulgence in attempts to concentrate specimens or to disclose bacilli in obviously unfertile locations such as the feces. The existence of a tuberculous lesion is determined by other corroborative clinical findings or by biologic verification with the second specific test for the presence of tubercle bacilli, *viz.*, simple culture methods, which have almost displaced the more expensive method of inoculation of guinea pigs for this purpose. These biologic tests should be secured from misinterpretation by a check for the characteristics of tubercle bacilli. Unlike the test for

acid-fast bacilli, which is relatively quickly performed, tests for tubercle bacilli require time, although the technical procedures of the culture method are simple. The third method of specific diagnosis is not new as such. It has been much abused in the past, particularly in regard to its proper performance. Now two reliable methods are available for testing the presence of tuberculin allergic hypersensitiveness in a patient. The older of these two methods, the intracutaneous (Mendel-Mantoux) test, is disadvantageous because of the injection method of application, the frequent necessity for several injections with different dilutions of tuberculin, and the caution which must be exercised when testing the tuberculous. Most of these disadvantages are overcome by the recently devised transcutaneous autolytic tuberculin test (*J. Lab. and Clin. Med.*, **29**: 398-403, 1944; *Am. Rev. Tuberc.*, **48**: 443-452, 1943; *Ibid.*, **54**: 159-165, 1946; *Tuberculology*, **8**: 72-77, 1946; *Ibid.*, **8**: 55-59, 1946) which obviates the use of the syringe and needle. Direct application is made on the skin of one strength tuberculin, incorporated in a transparent bland adhesive. It is applicable to any person, tuberculous or not, because of the self-limiting nature of the reaction it may induce, and can be applied repeatedly without possibility of mental anguish or of allergic sensitization by subsequent skin testing.

A widespread use of these three simple specific tests, when indicated, should not only aid the diagnosis and control of tuberculosis, but should extend the certified diagnosis of this disease into every field of medicine.

H. J. CORPER

BOOK REVIEWS

Medical Research. A Symposium. Edited by AUSTIN SMITH, M.D., Secretary, Council on Pharmacy and Chemistry; Director, Therapy and Research, A. M. A. Authors: WALTER C. ALVAREZ, M.D.; MILTON G. BOHRD, M.D.; EDON M. BOYD, M.D.; HERBERT O. CALVERY, Ph.D.; S. DEWITT CLOUGH; MORRIS FISHBEIN, M.D.; H. LOU GIBSON; TORALD SOLLMANN, M.D., and AUSTIN SMITH, M.D. 169 pp., 17 illus., 10 in color. \$5.00. Philadelphia: J. B. Lippincott Company, 1946.

Trying to define research is like trying to define God. The best definition is that it is an ideal. Here is an attempt to define medical research, and every individual who reads it will differ in evaluation. Most of the authors have written much and, therefore, in some circles, may be considered authorities. However, Sollmann preaches curtailment of voluminous writing: "work much, print little." If you have time, read it; but the best on research was written by Claude Bernard and Maurice Arthus in brief terms, like the Gettysburg Address. To Bernard and Arthus, the promise of millions meant little, and Austin Smith's figures of 69 to 720 million dollars contracted for research would have meant little. The trend of industry and government to take over medical research may spell suppression of individual enterprise, which stands paramount and is the pivot of Americanism as well as idealism. There will never be a mold for idealism, for truth, or for research because such men as Bernard, Arthus, and Laennec were individualists. As Bernard noted, the usual processes of research go in spiral cycles: (1) it begins with the observation of a fact; (2) an idea takes form as to the relation or meaning of the fact; (3) an experiment or observation is planned to test the idea; (4) this leads to the observation of new facts; (5) new ideas take form; and so we are off on a second cycle of observation, reflection, and verification.

Facts are the food of knowledge and, as with other food, both starvation and overstuffing hinder healthy activity. The Muse of invention comes to him who deserves her, not to him who merely waits. "Seek and ye shall find." What matters chiefly is to drive ahead and not to dawdle, and here the old rules of Descartes are helpful. An investigation that is not carried through to its logical consummation is apt to be more of a hindrance than a help to science. Whether a research be modest or pretentious, it must meet the strict criteria of scientific evidence. The observations themselves must be planned with proper controls and be sufficient in number to discount accidental variations within the chosen limits. Research workers do not spring fully trained from the head of any Jove. One can hardly agree that the laboratories of industry and of the government are often necessarily restricted to problems of more immediate practical application. Certainly ideals are not so limited as to exist only for individuals but not for industries or governments made up of individuals. What about the slogans of industry and government? Are they empty words?

Clough supplies the touch of the pharmaceutical house to the book "where the benefit of medical research may be said to culminate," with elucidating revelations. In spite of the revelation that the teachers need not necessarily be researchers, Boyd presents an elucidating chapter on the university medical school with a criticism of some editors who insert their personalities into the authors' papers. He also offers a criticism of the cumbersome, inadequate, redundant, and obsolete publication and book. Six disadvantages to a university professorship are listed. Alvarez champions the physician who will remain curious, and Fishbein briefly states that every institution devoted to scientific research must take account today of the mechanism by which the results of research are made public. Finally, Bohrod and Gibson indulge liberally in using one-fifth of the space on photography, reminding one of the x-ray technician who pictures everything in sight with stress on *graph* instead of *ology*, the latter obviously being most important in research.

To the research man of some experience, this book contributes little; to the novice and layman, it will find a place with de Kruif and many other modern popular books. It will interest them and therefore fills a place.

Denver, Colorado

H. J. CORPER

Practical Malariology. By PAUL F. RUSSELL, M.D., Colonel, MC, AUS, Parasitology Division, The Army Medical School, Field Staff, International Health Division, Rockefeller Foundation (on leave); LUTHER S. WEST, Ph.D., Head of Biology Department, Northern Michigan College of Education, Major, SnC, AUS (Reserve); and REGINALD D. MANWELL, Sc.D., Professor of Zoology, Syracuse University, New York. 684 pp., 238 illus., 8 in color. \$8.00. Philadelphia: W. B. Saunders Company, 1946.

This book gives clinical, laboratory and field information about malaria. Little is contributed to our knowledge of the clinical aspects of the disease. The chapter on therapy is important for its discussion of the value of atabrine and quinine in the treatment of this disease. Data on therapy, accumulated during the war, and hitherto unavailable for publication, are included in the book. The authors, in discussing the pathologic changes occurring in the disease, have emphasized the rôle that anoxia may play in the pathogenesis of the acute forms of malaria. Scanty support is given to the opinion of "vascular occlusions as the result of thrombi." The brief discussion on immunity summarizes the present views, but one cannot fail to realize the lack of knowledge on this point.

The two chapters on morphology and physiology of the plasmodia give much data that may be difficult to find elsewhere. It is interesting to find that the authors have included a discussion of plasmodial infections in bats and in the buffalo.

A large portion of the book is devoted to mosquitoes, their morphology, taxonomy and life cycle. The value of this book is enhanced by the inclusion of a discussion of the epidemiology of malaria throughout the world. Preventive measures are presented as they apply both to military and civilian problems. The experience obtained by the senior author while field director of the International Health Division of The Rockefeller Foundation and Chief Malariologist of the Allied Commission in World War II is reflected throughout the portion of the book dealing with preventive measures. Anyone interested in this phase of malaria no doubt will find many valuable suggestions. The larvicide DDT is discussed.

The book contains a variety of excellent illustrations. The quality of the paper used in this issue is much better than that in some recent publications. The data included in the table of contents are well selected and conveniently arranged, thus providing access to specific information. The index is complete. References at the end of each chapter contribute to the usefulness of the book.

It is the opinion of this reviewer that the authors fulfilled in a most creditable manner their task as stated in the preface. "For some time there has been needed a book which would gather up the important features of malariology, integrating new developments with the old and presenting a well balanced account of this disease which, from the standpoint of prevalence, some authorities have called the most important in the world today." More information has been included in the 684 pages of *Practical Malariology* than may be found in any other single volume pertaining to this subject. The book is most highly recommended for everyone interested in any phase of malaria.

Little Rock, Arkansas

R. H. RIGDON

La Cultura In Vitro Del Midollo Osseo. Problemi Di Fisiopatologia Ematologica Studiati Con La Tecnica Della Cultura Dei Tessuti. By AMINTA FIESCHI and GIOVANNI ASTALDI. 309 pp., 132 illus. 1000 lire. Pavia: Tipografia Del Libro, 1946.

Although a number of monographs dealing with sternal puncture have been published in the past twelve years, this monograph by Fieschi and Astaldi is the first of its kind in which such detailed studies of cultures of human bone marrow have been made. The senior author is known for his unexcelled monograph on sternal marrow studies, *Semeiologica de Midollo Osseo: Studio di Morfologia Clinica*, published in 1938. The monograph summarizes the results of nine years of study of bone marrow cultures and includes studies of bone marrow from normal persons and from patients with pernicious anemia before and after liver therapy, chronic myeloid and lymphatic leukemias, acute leukemia and Cooley's

anemia. The author utilized an original method of tissue culture derived from a modified Orgood's technic in which cultures were studied after fixation or in the fresh state, either unstained or stained, with supravital stains. In addition, in many cases the cultural behavior was observed under different gaseous conditions.

Cultures of normal bone marrow revealed that the normoblasts matured through the basophilic, polychromatic, orthochromatic and reticulocytic stages in approximately four days. Caryogenesis was observed both in the polychromatic and less frequently in the orthochromatic stage. Myelocytes lived about three days. Some myelocytes appeared to have phagocytic properties. The authors observed that the hemocytoblast, after originating from the "hemohistoblast" of Ferrata, is an irreversible element with myeloid function and lacks phagocytic properties. Eosinophils were the most resistant of the granulopoietic elements and lived *in vitro* up to ten or twelve days. The lymphocytes had a longer life span than the neutrophils, lacked phagocytic properties and did not show any tendency to develop into other cellular elements. There was a close relationship between histiocytes and monocytes. The megakaryocytes did not show any phagocytic activity or evidence of platelet formation. Plasma cells lived *in vitro* as long as ten or more days without degeneration. This finding was considered a point against the Pappenheim theory that the plasma cell represents a degenerated form. The plasma cell is derived from a plasmoblast which is believed to be of histoid origin. The authors deny the lymphocytic origin of plasma cells.

In cultures from patients with pernicious anemia it was noted that the megaloblasts progressively disappeared, either because of death of the cells, or through maturation. In some cultures many early megaloblasts passed through a micromegaloblastic stage to become cells of the normoblastic series, while later stages of the megaloblastic series (polychromatic and orthochromatic) died or matured to megalocytes. Following liver therapy, there was a rapid regression of histiocytic hyperplasia, pronounced regeneration of hemocytoblastic and myeloblastic-promyelocytic elements and tendency of the bone marrow to return to normal along the lines mentioned above. These investigators observed normal erythrocytes derived from megaloblasts. The continued action of liver extract *in vitro* when it had been administered *in vivo* was explained on the hypothesis that the antianemic principle is fixed on the marrow cells.

Bone marrow cultures from patients with chronic myeloid leukemia revealed that the hemocytoblast multiplied by cariocynesis and evolved along myeloid lines. Normal and abnormal types of myeloblasts were observed; the latter were never seen in normal bone marrow cultures. Disturbances in myeloid maturation were attributed to the presence of abnormal myeloblasts. Many myeloblasts matured normally but only a few functioned normally. The authors noted a disturbance in the maturation of leukemic lymphocytes while the myeloid cells in the lymphatic leukemic cultures maintained a normal and complete independence from leukemic cells. In acute leukemia the great majority of hemocytoblasts and myeloblasts consisted of pathologic cells unable, even *in vitro*, to assume a hemopoietic function.

In a case of Cooley's anemia it was concluded that the abnormality of maturation resides in the nucleus rather than in the cytoplasm of the cell. The tenth and final chapter deals with caryologic activity of marrow cells *in vitro* under different conditions, including the action of colchicine and penicillin.

This book is well printed and has numerous well selected and unexcelled photomicrographs and color plates. In some of the diseases the number of cases studied does not warrant so much discussion and definite conclusions; yet this work must be accepted as a remarkable contribution to our knowledge of marrow cultures and pathogenesis of certain blood diseases. It stresses again the importance of this method of study in a field where strict morphologic approach would probably be insufficient for an understanding of many blood dyscrasias.

Chicago

LOUIS R. LIMARZI

Manual de Patología Quirúrgica. Ed. 4, revised. By Dr. RAFAEL ARGÜELLES. Three volumes, 1900 pp., 1122 figs. Paper. Barcelona, Spain: Editorial Científico Médica, Imprenta Elzeviriana y Librería, 1945.

This book is hardly what the name implies. It is not a text of pathology but a manual of clinical and physical surgical diagnosis. Emphasis is given to the pathologic background not only for the diagnosis, but for the treatment of many surgical lesions. Written from the viewpoint of surgery, the book, as the author himself states, is for the medical student and those in general practice who must do some general surgery. It would interest neither the surgical specialist nor the pathologist. There is no histopathology in its more than one thousand figures. While many of the figures are excellent, they have lost much in having been badly printed on poor paper.

The material devoted to neoplasms, instead of being comprehensively grouped together, is widely scattered among the surgical topics and specific subjects are difficult to find.

Since the book is practical, the reader is not bothered by medical history and the presentation of conflicting opinions. The material is presented clearly and simply. A short bibliography is given at the end of each chapter and most of the references given are to German authors.

Cincinnati

W. McK. GERMAIN

Las Hipertensiones Arteriales. Ponencia en el Congreso Nacional de Cardiología. Dr. C. JIMENEZ DIAZ. 181 pp., 1 fig., 2 tables. Paper. Barcelona, Spain: Imprenta Claraso, Villaroel, 1945.

In this monograph the author has given a very complete review of the various theories of the etiology of hypertension and of its medical and surgical management. In referring to medical management, Diaz notes that strict diet has been abandoned since, instead of improving the patient's health, it often contributes to the deterioration of his general state. A small section is given to the analysis of the results that have been obtained with the use of renin.

The author follows the classification of Keith, Wagener and Barker in dividing hypertensives into four groups which are determined by their clinical manifestations. The monograph ends with a very large and comprehensive bibliography in which most of the authors are north Americans. The author has made no noteworthy original contributions.

W. McK. GERMAIN

Experimental Hypertension. By WILLIAM GOLDRING *et al.* 179 pp., 42 figs., 25 tables. \$3.75. New York: The New York Academy of Science, 1946.

The past two decades have seen profound alterations in our concepts of arterial hypertension. In addition to a refinement of the clinical aspects of this disease, its pathogenesis has been subjected to intense investigation by experts in various fields of biology. Although a complete understanding of hypertension is not yet apparent, many facts have been established and many chemical mechanisms have been elucidated, as a result of which proper treatment of hypertension has become more imminent.

This volume is a compilation of formal papers and the discussion of them which was presented in a conference on the subject held before The New York Academy of Science. In the formal papers are included summaries of data and the opinions of the foremost contemporary investigators in this field. The present status of the subject is reviewed and the directions in which future research will follow are outlined.

This book should be read by those who are interested in the experimental aspects of hypertension. It furnishes a starting point for those who may not have kept up with the reported literature. It also offers suggestions for work upon which future therapy will depend.

Detroit

S. D. JACOBSON

Experimentelle Grundlagen zu einer modernen Pathologie. Von der Zellular- zur Molekular-pathologie. By PAUL BUSSE GRAWITZ, Prof. Dr. med. 359 pp., 120 illus., 6 in color. 32 Swiss francs. Basel: Benno Schwabe and Company, 1946.

Grawitz had discarded Virchow's conception of the cell as the primary tissue unit ("omnis cellula e cellula") and has replaced it with a hypothesis that cells develop from acellular connective tissue. This idea is based on a large amount of experimental evidence, mainly, implantation of various tissues into subcutaneous pouches and a variety of other locations in rabbits, and subsequent histologic examination after varying intervals. The cornea of the rabbit was used for the basic experiments and, later, tissues which were damaged by physical and chemical agents. The transformation of the amorphous tissue matrix into cells results from the action of a factor produced by damaged tissues (for instance, secretion of wounds). When the tissue factor ceases to function, the process is reversed: the cells become transformed back into connective matrix. Invasion of cells from the tissues of the host was prevented, according to the author, by collodium bags.

Cohnheim's theory of inflammation which has been the basis of our entire modern thinking in this branch of pathology is thrown overboard together with Virchow's cellular pathology.

Grawitz's ideas seem to be the outgrowth of a family tradition, having been suggested by his grandfather, Paul Grawitz, and later elaborated by his father, Otto Busse Grawitz. Detailed description of technic and of experimental data occupies most of the volume. There are abundant illustrations.

The book is thought provoking and stimulating. The author expresses the hope that others will repeat his experiments and check the results. In this hope we join. Only unbiased, critical reinvestigation can permit a fair evaluation of this work which was published in 1945 in Spanish and of which an English edition has been announced by the author.

Chicago

I. DAVIDSOHN

Outlines of Internal Medicine. Five Parts. Ed. 5. Edited by C. J. WATSON, M.D., Head[†] Department of Medicine, University of Minnesota. Paper. \$9.00. Dubuque, Iowa: William C. Brown Company, 1946.

Contrasted with the customary course of didactic lectures and their weighting with excess detail and coloring, these outlines adhere strictly to the business of teaching the student the fundamentals of internal medicine. The method used, while pointed and direct, allows greater opportunity for clinical observation of patients with direct application of the basic knowledge. As is to be expected in a work of this kind, the material has been thoroughly sifted, freed of controversy in so far as possible, brought up-to-date and presented in practical fashion. Especially outstanding is the section in Part II on diseases of the liver and biliary tract and jaundice by Dr. Watson.

Inclusion of a select list of references for collateral reading serves as a constant reminder that there are other opinions besides those of the instructor, and that it is wise to investigate these for a more complete viewpoint. The arrangement in loose-leaf form permits addition of new material as it is developed and accepted in medical practice, lending flexibility to the presentation of the subject and making the outlines valuable for current use as well as review purposes.

Detroit

William S. Reveno

The Diagnosis and Treatment of Bronchial Asthma. By LESLIE N. GAY, M.D., Assistant Professor of Medicine of the Johns Hopkins University School of Medicine, and Director of the Allergy Clinic of the Johns Hopkins Hospital. 334 pp., 80 figs., 39 tables, 4 plates. \$5.00. Baltimore: The Williams & Wilkins Company, 1946.

The author, who has devoted more than twenty-five years to the study of bronchial asthma and its diagnosis and treatment, is Director of the Allergy Clinic of the Johns

Hopkins Hospital and one of the country's authorities on this subject. From the wealth of his experience as a practitioner, teacher and investigator in this speciality, he has produced a book which provides the student and the practitioner of medicine with an adequate understanding of, and a proper approach to, this poorly understood disease. The author stresses the need for a detailed history and a careful examination of each individual patient, and cautions against indiscriminate use of diagnostic procedures and undue reliance upon some of them. He devotes a chapter to the pathology of bronchial asthma, with a brief summary of published cases and a review of 24 cases with autopsy at the Johns Hopkins Hospital. This chapter, in itself, is a valuable contribution to the knowledge of this disease. The diagnosis and the differential diagnosis of bronchial asthma are thoroughly presented. A chapter on psychosomatic asthma is convincingly and interestingly written. A chapter on the treatment of the disease is a master-piece. It is thorough, practical and up-to-date, and reflects the author's extensive and successful experience.

This book should be included in the library of every medical practitioner as a ready reference.

St. Paul, Minnesota

KANO IKEDA

Amino Acid Analysis of Proteins. By WILLIAM H. STEIN, REGINALD M. ARCHIBALD, ERWIN BRAND, R. KEITH CANNAN, HANS T. CLARKE, JOHN T. EDSALL, G. L. FOSTER, STANFORD MOORE, DAVID SHEMIN, ESMOND E. SNELL and HUBERT B. VICKERY. Vol. 17. Art. 2. 183 pp., 11 tables. \$2.25. New York: The New York Academy of Sciences, 1946.

This publication consists of a series of papers presented at a Conference on Amino Acid Analysis of Proteins held by the Section of Physics and Chemistry of The New York Academy of Sciences, October 1945. The topics of the papers include methods of analyzing amino acids in protein hydrolyzates by means of specific precipitants, isotope dilution, chromatograph and ion exchange, microbiologic approaches, and by specific enzymes.

Philadelphia

F. WILLIAM SUNDERMAN

Die Scheuermannsche Krankheit und ihre Differentialdiagnose. By J. E. W. BROCHER, Privatdozent an der Universität Genf. 91 pp., 122 illus. Basel: Benno Schwabe & Co., 1946.

Juvenile kyphosis, first described in 1920 by Scheuermann, is not well enough known by physicians in spite of its great frequency.

This instructive monograph gives information about the clinical symptoms, differentiation from more serious diseases of the spine, x-ray findings and treatment. In regard to the pathogenesis, the author does not accept the view held by Schmorl who studied the anatomic findings in late stages of the disease and believed that protrusion of the intervertebral disks into dorsal vertebrae is the underlying cause of Scheuermann's disease.

Two photomicrographs and 118 prints of x-rays illustrate this precise text.

Wichita, Kansas

C. A. HELLWIG

Vitamine, Hormone Fermente. Ed. 3. By RUDOLF ABDERHALDEN, Dr. med., Dozent. 250 pp. 14.50 Swiss francs. Basel: Benno Schwabe & Co., 1946.

This volume, the third edition since 1943, covers vitamins, hormones and enzymes which are treated as a functional unit. Emphasis has been placed on the chemical and physiologic properties, while only the principles of the laboratory tests are given. The clinician will appreciate the listing of proprietary names of the respective drugs from Swiss and German companies and their composition.

The clinical pathologist will miss a complete bibliography as well as a detailed description of the technic of laboratory tests.

C. A. Hellwig

Dynamische Reaktionspathologie. By K. v. NEERGAARD, Professor, Direktor der Klinik und Poliklinik für physikalische Therapie an der Universität Zürich. 307 pp., 26 figs., 24 Swiss francs. Basel: Benno Schwabe & Co., 1946.

This book is an attempt to show that the recent developments in the philosophy of science, notably the Heisenberg principle of uncertainty, will change greatly the foundations of medical science. The author challenges the view that medicine is applied biologic science and that pathologic processes follow chemical or physical laws. The cause-and-effect assumption has no place in v. Neergaard's new system of a theoretical "autonomous" medicine. Disease is the reaction of the living organism against injuries and the individual reaction is as a rule unpredictable. In the treatment of disease emphasis should be placed more on the type of reaction than on etiologic factors.

The practical conclusions in regard to medical education and practice form the major part of the book. According to the author, clinical observation at the bedside, intuition and active therapy, especially physiotherapy, should again take the leadership from pathology and experimental medicine with its emphasis on etiology. Medical schools should educate general practitioners and the latter should be qualified to treat 90 per cent of the diseased.

Many may disagree with v. Neergaard's conception. His book is significant, however, because it portrays the crises and conflicts of contemporary physicians in Europe.

C. A. HELLWIG

Renal Hypertension. By EDUARDO BRAUN-MENNÉNDEZ, JUAN CARLOS FASCILO, LUIS F. LELOIR, JUAN M. MUNOZ, ALBERTO C. TAQUINI, Institute of Physiology, Faculty of Medical Sciences and Institute of Cardiology, V. F. Grego Foundation, Buenos Aires, Argentina. Translated by LEWIS DEXTER, M.D., Harvard Medical School and Peter Bent Brigham Hospital, Boston. 451 pp., 91 figs., 5 tables. \$6.75. Springfield, Illinois: Charles C Thomas, 1946.

This volume, attractively printed and well illustrated, is the result of years of investigation by disciples of Houssay, the physiologist. The Argentinian edition was published in 1943. The present translation, which is of high literary quality, incorporates additions in text and bibliography by the translator, himself a former student of Houssay. The material includes much that is universally accepted, as well as features of controversial status. Each chapter ends with a brief statement of its contents and conclusions.

There is a judicious weighing of the significance of the anatomic states frequently found in hypertensive persons, and of surgical methods employed in therapy. The recent report of Trueta and his associates (*Lancet* 2: 237-238, 1946) may further emphasize the rôle of neurosurgery. In it is this last sentence, "Finally, we believe that hypertension may be the result of disordered activity of some nerve center or centers from which impulses are sent to the same vascular effector-organs as are involved in the [renal] cortical ischemia and medullary congestion described by us."

For the general pathologist, the volume of Braun-Menéndez and his group serves as an excellent reference volume, when used in conjunction with such a work as the recent monograph, "Renal Diseases," by E. T. Bell.

Fort Wayne, Indiana

S. M. RABSON

Intracranial Complications of Ear, Nose and Throat Infections. By HANS BRUNNER, M.D., Associate Professor of Otolaryngology, University of Illinois College of Medicine. 444 pp., 95 figs. \$6.75. Chicago: The Year Book Publishers, Inc., 1946.

This book of 444 pages, including the index, is divided into two sections. Section I, General Introduction, deals with the anatomy and physiology of the brain, its various spaces, sinuses and coverings and the spinal fluid, and consists of 82 pages. Section II, Clinical Aspects, comprises the remaining 376 pages.

The text is adequately illustrated, especially Section I. The illustrations consist of schematic drawings and photographs of anatomic and pathologic specimens and are especially striking because of their clarity, simplicity and excellent labeling.

The anatomy and physiology is written in such a manner that the reader readily understands why various normal and abnormal processes occur when, formerly, they just happened with no logical explanation.

Section II on Clinical Aspects is a thorough discussion of the pathology, symptomatology, prognosis and treatment of every possible intracranial complication of ear, nose and throat infections. Although recent use of the sulfa drugs and antibiotics has greatly reduced the incidence of these complications, there remains a great need for such a compilation as this to keep us alert to the possible hazards of ear, nose and throat infections. The pathologist who is called upon to conduct clinico-pathologic conferences will find this text a valuable reference.

The excellent organization of the subject matter, the ease of expression and technical thoroughness reflect the character and talents of the author.

This is a work the otolaryngologist needs and is eager for; the neurologist, neurosurgeon and pathologist can profit greatly by it. It is a valued addition to any medical library.

Detroit

JAMES E. CROUSHORE

The Problem of Lupus Vulgaris. By ROBERT AITKEN, M.D., F.R.C.P.E., F.R.S.E., Lecturer in Diseases of the Skin, Edinburgh University, Physician to the Skin Department, The Royal Infirmary of Edinburgh. 69 pp., 31 figs., 19 in color. Baltimore: The Williams & Wilkins Company, 1946.

This small book is written primarily as a plea for more effective treatment of those afflicted with this serious and disfiguring disease. The author particularly emphasizes the value of ultra-violet irradiation with the Finsen-Lomholt lamp. Another chapter is devoted to the tuberculin treatment of lupus vulgaris which the author believes does not deserve the disrepute into which its use has fallen. The incidence of lupus vulgaris is much higher in Scotland and especially in northern Europe than in the United States, and its treatment offers a serious and neglected problem. The illustrations are unusually good and the discussion of treatment is excellent.

It is to be regretted that the writing of this book evidently just preceded the introduction of the "Charpy" treatment (France) of lupus vulgaris with irradiated Ergosterol (D-2) plus calcium, or without the latter (Calciferol) as employed in England. The results to date with this method of treatment have been so effective that all the older methods of treatment will probably become obsolete.

Detroit

L. W. SHAFFER

Essentials of Clinical Proctology. By MANUEL G. SPIESMAN, M.D., Proctologist, Mount Sinai and Edgewater Hospitals, former Head of Cook County Hospital Rectal Clinic, Chicago. 238 pp., 62 illus. \$4.00. New York: Grune & Stratton, 1946.

This book on proctology is practical and to the point. It is compact enough to be read from cover to cover. The printing is good and the book is well illustrated. There is an adequate discussion of such common rectal conditions as hemorrhoids, anal fissure and pruritis ani, with directions for home, office and surgical treatment. There is a discussion of pectinosis and pectonotomy (a surgical approach to the treatment of tight and painful anus).

According to the author, adenoma is the commonest tumor of the rectum and colon; polyps are forerunners of carcinomas of the rectum and colon in from 40 to 50 per cent of cases; polyps proceed from benignancy to malignancy; the development of carcinoma in a polyp depends on the rate of growth of the latter; in a slowly growing polyp, carcinoma will probably not develop, whereas in a rapidly growing polyp it is only a question of time before carcinoma will appear.

Detroit

S. G. MEYERS

Psychiatric Interviews With Children. Edited by HELEN LELAND WITMER, Ph.D., Smith College School for Social Work. 443 pp. \$4.50. New York: The Commonwealth Fund, 1946.

This book is a symposium by nine competent contributors. Its purpose is to provide material to students and practitioners of psychiatry for the perfecting of their own technics in the psychotherapy of children. Ten case histories are given to illustrate the significant words and actions and the underlying reasons by which the therapists achieved a satisfactory solution of their patients' problems. The book affords delightful reading and is highly instructive for anyone interested in problems of human relationships.

Eloise, Michigan

MILTON H. ERICKSON

OBITUARIES

IVO AMAZON NELSON

Ivo Amazon Nelson who, with his brother, founded the Nelson Clinical and Pathological Laboratories of Tulsa, Oklahoma, died October 26, 1946, at the age of 52.

Born in Para, Brazil, Dr. Nelson received his medical degree from Oklahoma University Medical School and served as staff pathologist at St. John's Hospital in Tulsa from 1927 to 1945, when ill health forced his retirement.

He was a member of the Oklahoma Association of Pathologists and of the Oklahoma State Cancer Society. He became a member of the American Society of Clinical Pathologists in 1928 and served as Counselor for Oklahoma from 1932 to 1939, on the Board of Censors from 1935 to 1941, and as a member of the Board of Registry of Medical Technologists from 1942 to 1944.

PAUL ROTH

Paul Roth, of Battle Creek, Michigan, died November 7, 1946. He was born in Switzerland and came to the United States at the age of 16. A graduate of the American Medical Missionary College, he pioneered in the use of oxygen for medical treatment and in testing basal metabolism. He was a member of the Michigan State Medical Society and joined the American Society of Clinical Pathologists in 1924.

NEWS AND NOTICES

SOCIEDAD CUBANA DE MÉDICOS LABORATORISTAS CLÍNICOS

Dr. Antonio Sellek, president of the Sociedad Cubana De Médicos Laboratoristas Clínicos, who was elected a corresponding member of the American Society of Clinical Pathologists at the annual meeting in San Francisco in June 1946, has advised the editor that his Society has accomplished the following:

1. Recognition has been obtained from the Commission on Salaries of the Minister of Labor that the physician engaged in clinical pathology is a specialist who should have all economic opportunities given those devoted to other specialties of medicine.

2. A resolution has been dictated by the Director of Health stating that the physician is recognized as the only professional man who may direct a clinical laboratory. Violations of this ruling are to be reported to the judicial authorities and considered an illegal practice of medicine and a crime against Public Health.

3. A commission has been appointed to outline the functions of physicians practicing in clinical laboratories in Cuba.

Dr. Sellek also reported on the Eighteenth Congress of the Pan-American Medical Society in Havana, December 3 to 8, attended by delegates from 19 countries including the United States, which was represented by members of the American Medical Association. At this meeting, the Medical Charter of Havana was promulgated which declared that: each individual in America has the right to enjoy health; each physician in America has rights with respect to liberty and exercise of the professions; it is a fundamental obligation of all organizations of American physicians to maintain as a basic point in their programs the right of freedom in professional practice.

The Congress also passed a resolution, introduced by Dr. Sellek, stating that it is officially recognized that direction of the clinical laboratory is a branch of medicine which should be practiced exclusively by physicians.

THE MINNESOTA SOCIETY OF CLINICAL PATHOLOGISTS

The Minnesota Society of Clinical Pathologists has gone on record as favoring the use of existing clinical laboratory facilities for Rh determinations and tissue diagnoses. The Society has recommended to the Minnesota State Medical Society that the existing clinical laboratory facilities be used rather than those of the laboratory of the State Board of Health.

The Society also has appointed a committee to investigate the facilities of the Northwest Institute of Medical Technology and the Minnesota School of Commerce, both of Minneapolis, and to render a report on its findings and recommendations in connection with veterans' training under the G. I. Bill of Rights.

Arrangements are being made to give the first of the recently established lectures in honor of Dr. A. H. Sanford at the Annual Session of the Minnesota State Medical Association in June 1947.

The officers of the Society are Dr. Kano Ikeda, St. Paul, president; Dr. George L. Merkert, Minneapolis, vice-president; and Dr. Gilbert Kvitrud, St. Paul, secretary-treasurer.

THE COLORADO SOCIETY OF CLINICAL PATHOLOGISTS

The last regular meeting of the Colorado Society of Clinical Pathologists was held January 18, 1947, at the Cosmopolitan Hotel in Denver.

Dr. J. B. McNaught reported on the College of American Pathologists and a symposium was held on brucellosis, with Dr. Miriam Benner in charge. Dr. Charles Davis, Dr. Enid K. Rutledge and Dr. Robert W. Gordon led the discussion.

The next meeting of the Society is scheduled for April 19, 1947.

PERSONALS

Dr. Walter M. Simpson was recently awarded the following citation by Admiral William F. Halsey for services in the Medical Corps of the United States Naval Reserve:

"For meritorious performance of duty while serving as Chief of Medicine and later as Executive Officer of the first advanced naval base hospital to be established in the South Pacific area during the period from April 12, 1942 to November 1, 1943. Captain Simpson reflected great credit on himself by his outstanding professional ability, leadership and keen judgment. As liaison officer he dealt with officials of foreign nations with tact and diplomacy, thus contributing materially to the harmonious relations with foreign nationals. As President of the Malaria Control Commission, and Sanitary Commission, he organized and initiated the measures which led to the control of malaria and other tropical diseases. His courageous conduct was in keeping with the highest traditions of the United States Naval Service."

Dr. Louis E. Marshall, formerly of the Army Institute of Pathology, Washington, D. C., was appointed Director of Laboratories at the House of the Good Samaritan Hospital in Watertown, New York, beginning January 1, 1947.

CARDIAC LESIONS IN SUBACUTE BACTERIAL ENDOCARDITIS TREATED WITH PENICILLIN

REPORT OF NINE CASES*

EUGENE HILDEBRAND, M.D., AND WALTER S. PRIEST, M.D.

*From the Department of Pathology, Northwestern University Medical School, and the
Departments of Internal Medicine, Northwestern University Medical School
and Wesley Memorial Hospital, Chicago, Illinois*

The efficacy of penicillin therapy in the treatment of subacute bacterial endocarditis is well established. In a series of 34 patients treated by one of us,¹⁵ there were 12 deaths and autopsy was performed in 10 of the 12 subjects. In each instance, the diagnosis of subacute bacterial endocarditis was established by typical physical findings and repeated positive blood cultures and was confirmed at autopsy.

"Standard mitral" and "standard tricuspid" sections were taken according to the method of Gross.⁹ Additional sections of valvar and mural endocardial lesions were taken as indicated. Serial sections of the myocardium were not made, but in the sections of valves generous portions of the adjacent myocardium were included. These sections were stained with hematoxylin and eosin and by the method of Brown and Brenn,² and stains for calcium were made in special cases according to the method of Baecher.¹¹

Detailed study of approximately 450 microscopic sections of this autopsy material disclosed pertinent data regarding possible reasons for failure of penicillin therapy, data regarding pathogenesis and mechanism of healing and evidence of myocardial involvement. Thus, we obtained a better understanding of the clinical course of subacute bacterial endocarditis under penicillin therapy. Clinical data are briefly correlated with the anatomic findings. Only positive anatomic findings are presented and, in general, the pathologic anatomy here described is limited to the heart.

REPORT OF CASES

Case 1

Clinical data. The patient was a woman, aged 36, who had been ill for eight months prior to therapy. A previous history of rheumatic fever was doubtful. She received an average of 200,000 units of penicillin per day intermittently over a period of seven months. *Streptococcus viridans* was recovered from the blood. *In vitro*, its sensitivity to penicillin was 0.02 units per cc. During the numerous courses of interrupted intramuscular treatment with penicillin, the blood culture was occasionally positive. Cultures were taken almost every day. During continuous intravenous "drip" therapy with penicillin, a negative blood culture was obtained on all occasions. Death occurred as a result of bowel hemorrhage, during a course of penicillin therapy in which heparin was administered.

*Received for publication, February 8, 1947. Penicillin used in this study was supplied in part by Commercial Solvents Corporation, Lederle Laboratories and Schenley Laboratories, all of New York, N. Y., and by the National Research Council and Northwestern University.

Gross findings. The heart weighed 238 gm. There was a slight degree of diffuse epicardial fibrosis. The right ventricle was slightly hypertrophied and dilated. Moderate thickening of the free edge of the mitral valve was observed and to the valve, near the interventricular septum, there was a firmly attached, slightly lobulated vegetation measuring 8 by 7 by 2 mm. which involved both the auricular and ventricular portions of this part of the valve cusp. A second vegetation of the anterior leaflet measuring 7 by 4 by 3 mm. was found which appeared to be partly covered by fibrosed endocardium. To the right and left of this lesion were multiple minute vegetations at the free margin of the valve, the largest measuring 4 by 2 by 1 mm. All vegetations were firmly adherent to the valve cusp and were covered by small amounts of fibrin.

Microscopic findings. In a standard mitral section, the valve was scarred and contained many capillaries. In a section taken at the site of the first *vegetation* described grossly, the latter surrounded the distal portion of the valve. The vegetation was found to be composed of much fibrin with enmeshed, intact or fragmented red blood corpuscles, occasional masses of polymorphonuclear neutrophils, scattered mononuclear cells and occasional clumps of bacteria in varying stages of disintegration. Blood cultures taken nearly every day for thirty days prior to death, had all been negative. At the base of the vegetation, there was a small amount of granulation tissue. In this granulation tissue, a capillary was found to be completely filled with bacteria (Fig. 16). This observation was confirmed by the Brown and Brenn stain. In a *standard tricuspid section*, the valve was slightly scarred, but the endocardium was intact. In one section of cardiac muscle, a fusiform area of abscess formation was noted immediately adjacent to, but not surrounding, an arteriole (Fig. 17). The cells of this infiltrated area were mainly lymphocytes, but there were also scattered, irregularly outlined, large cells with densely staining basophilic cytoplasm. Typical fibroblasts infiltrated all margins of this area and seemed to merge distally with the immediately adjacent fibrous tissue. No typical Aschoff cells were noted. In another section, a capillary, surrounded by a few lymphocytes, was found to be filled with fibrin. No bacteria were found in this capillary or in the adjacent tissues.

Case 2

Clinical data. The patient was a woman, aged 46, who gave a history of previous rheumatic fever and had been ill for twenty-six weeks before therapy was instituted. She received an average of 200,000 units of penicillin daily for twenty-five days. *Streptococcus anhemolyticus* was recovered from the blood; its sensitivity to penicillin *in vitro* was 0.02 units per cc. Blood cultures became negative within twenty-four hours after therapy was instituted and remained negative. Death from massive pulmonary atelectasis occurred on the twenty-fifth day of therapy.

Gross findings. The heart weighed 500 gm. Two vegetations, the larger measuring 1.5 cm. in diameter, were seen attached to the left side of the base of the pulmonary artery (Fig. 2). They appeared to be attached to an atheromatous plaque. Several other firm vegetations on the ventricular surface of the aortic cusps were markedly shortened verti-

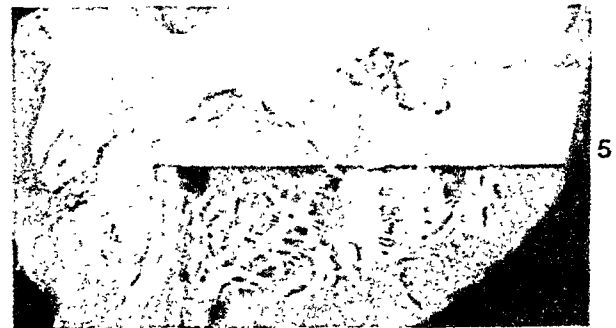
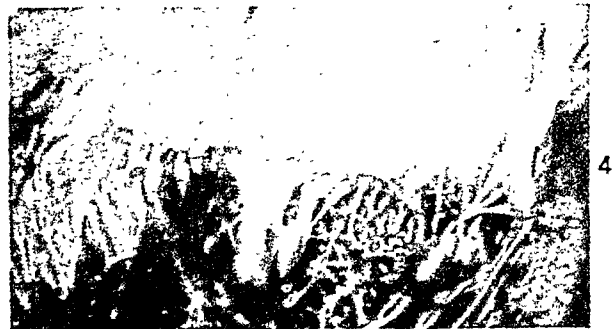
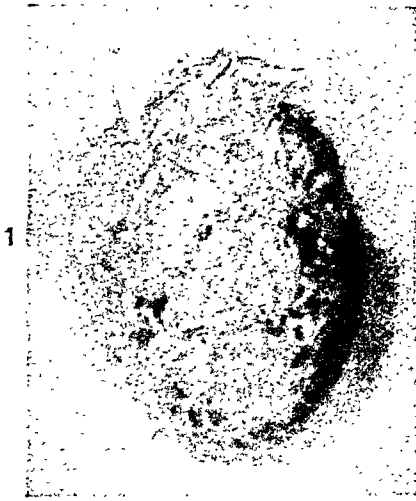
FIG. 1 (Case 9). Acute fibrinous pericarditis and multiple petechial hemorrhages. The blood culture was negative prior to death. Dicumarol had been administered to the patient.

FIG. 2 (Case 2). Fresh vegetations at the base of the pulmonary artery. Patient had received injections of heparin with penicillin.

FIG. 3 (Case 9). Marked destruction of aortic valve by ulceration and calcification in spite of 19 weeks of treatment with penicillin and blood penicillin levels 2.5 times the sensitivity of the organism *in vitro*. Microscopically, there were thin layers of bacteria-free fibrin overlying the ulcerated areas.

FIG. 4 (Case 5). Healed lesions of the mitral valve. Some of the chordae tendineae are calcified, others have ruptured. No anticoagulants were administered to the patient with the penicillin.

FIG. 5 (Case 4). Vegetations grossly showing advanced stage of healing, but microscopically (see Fig. 11) still showing fibrin laden with bacteria and acute inflammatory reaction.



cally. Slight thickening of the margins of the mitral valve cusps was noted, but no vegetations were seen.

Microscopic findings. A section of the aortic valve lesion showed almost complete destruction of the cusp with replacement by a large vegetation which was composed mainly of fibrin and masses of partly disintegrated bacteria. At the base of this vegetation was a large amount of granulation tissue containing many polymorphonuclear neutrophils. These acute inflammatory cells were limited to this area and did not extend into the overlying fibrin in which the bacteria were present. The endocardium at the base of the vegetation was partly intact and beneath the endocardium was a localized thin layer of fibroblasts and mononuclear cells. An occasional eosinophil was seen, but no polymorphonuclear neutrophils were present. A section of the lesion of the *pulmonary artery* showed changes which closely resembled those described above.

Except for the polymorphonuclear neutrophil reaction, the vegetations of the aortic valve and pulmonary artery were typical of those encountered prior to penicillin therapy. Yet, heparin was used and frequent blood cultures were all negative during the entire course of penicillin therapy.

Case 3

Clinical data. The patient was a woman, aged 33, who gave a history of previous rheumatic fever and of having been ill for five months prior to penicillin therapy. Cultures of blood were positive for *Streptococcus viridans*; *in vitro*, the sensitivity of this organism to penicillin was 0.04 unit per cc. The patient received an average of 200,000 units of penicillin daily for twenty-nine days, which resulted in apparent clinical and bacteriologic cure. She felt well and her temperature was generally normal but occasionally rose to 100 F. Following treatment, she remained under observation for forty days, during which period she had four episodes of minor embolism, the sedimentation rate persisted between 30 and 40 mm. per hour, and 15 blood cultures were taken, all of which were negative. She was readmitted eleven weeks after completion of the first course of therapy and four weeks after discharge from the hospital with unmistakable clinical signs of active subacute bacterial endocarditis. Repeated blood cultures were negative. She was given 300,000 units of penicillin daily for twenty-six days. *Streptococcus viridans* was recovered from the blood two days before death; its sensitivity to penicillin, *in vitro*, was 0.04 unit per cc. Death, due to congestive heart failure, occurred six months after the initial therapy with penicillin and five weeks after the second course.

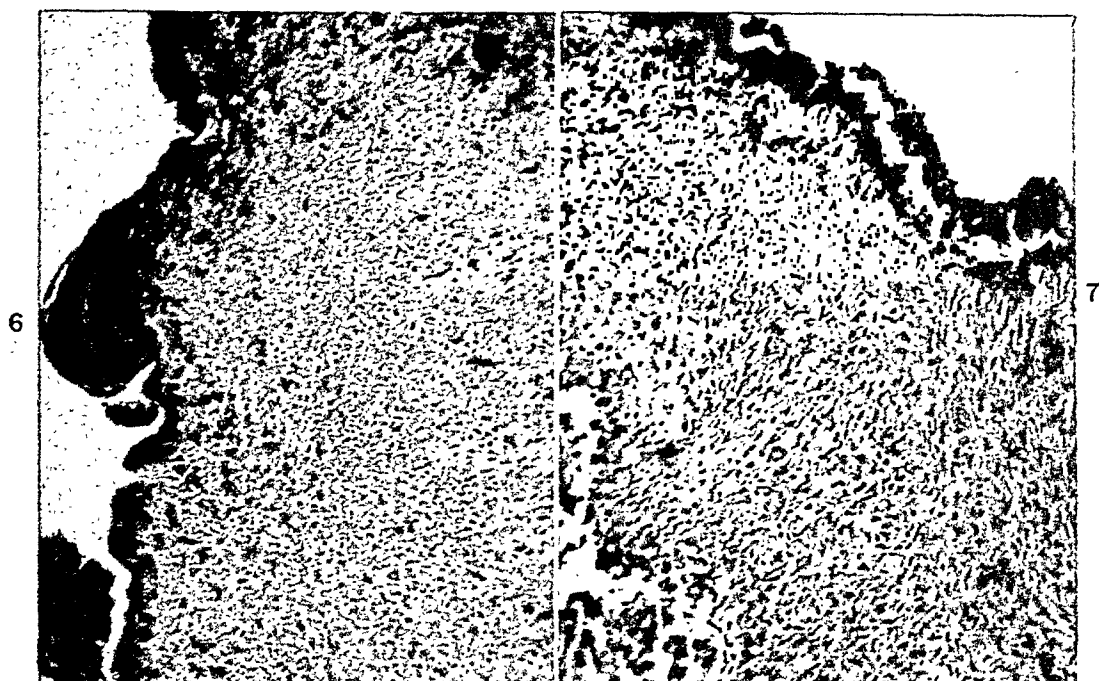
Gross findings. The mitral valve was markedly scarred. Several minute, firm vegetations, apparently covered by endocardium, were attached to the margins of the leaflets. Two vegetations were attached firmly to the chordae tendineae. Some scarring of the auricular endocardium was present and areas of roughening and infiltration extended upward on the left side of the left auricle for a distance of several centimeters. In this region were noted a few small areas of ulceration surrounded by marked scarring. The other valves showed no vegetations.

Microscopic findings. The standard mitral section showed diffuse thickening and scarring of the valve. Much of the scar tissue was hyalinized and relatively avascular, but a few capillaries were found. The auricular surface of the valve, a short distance from its free

FIG. 6 (Case 5). Thin layer of organizing fibrin with regenerating endothelium. The regenerating endothelium showed up well in the slide, but is not shown well in the figure.

FIG. 7 (Case 5). Minute area, devoid of endocardium, covered with a thin layer of bacteria-free fibrin (right upper portion of field); fibroblastic invasion of the fibrin and underlying valve substance; and (in the left lower corner) marked calcification. Blood cultures were negative prior to death.

FIG. 8 (Case 5). Low power photomicrograph of a portion of a small branch of the pulmonary artery showing one of two true vegetations undergoing organization.



margin, was the site of a small, slightly elevated, irregular vegetation. Its size was approximately one-half the diameter of a high power field and it consisted of masses of bacteria and fibrin. Within the underlying valve substance, there were a few fibroblasts and capillaries, but no polymorphonuclear neutrophils. A short distance distally, at the free margin of the vegetation, were masses of bacteria which seemed to surround a chorda. Polymorphonuclear neutrophils were seen in the vicinity of the bacteria. A section from the area of ulceration of the *left auricle* showed absence of part of the endocardium. A vegetation in this area contained clumps of bacteria superficially enmeshed in fibrin. The underlying granulation tissue contained many mononuclear cells and plasma cells and an occasional polymorphonuclear neutrophil. The *standard tricuspid section* showed scarring and thickening only. Bacteria were found in a postmortem mural clot adjacent to this valve.

Case 4

Clinical data. The patient was a man, aged 65, who gave no history of previous rheumatic fever and was ill for two months prior to penicillin therapy. *Streptococcus viridans* was recovered from the blood; *in vitro*, its sensitivity to penicillin was 0.8 unit per cc. He received from 200,000 to 300,000 units of the drug daily for sixty days, during which time transitory encephalitis and Broca's aphasia developed. Death was apparently due to uremia.

Gross findings. The heart weighed 390 gm. Its consistency was generally decreased and the myocardium was brown in color. The endocardium, except for the valves to be described below, appeared normal. The mitral valve ring measured 13 cm. in circumference (Fig. 5). The free border of the valve was moderately thickened and contained three nodular elevations, the largest of which measured 1 cm. in diameter. These were irregular in shape, friable, red in color at the distal end, shading to light yellow at the bases and extended to involve a small portion of the adjacent auricular and ventricular surfaces of the valve. The bases of the lesions appeared scarred. The slightly scarred tricuspid valve revealed an area of ulceration measuring approximately 0.5 cm. in diameter on the auricular surface of the septal leaflet. The ulcer was covered with two minute red verrucose projections, the larger of which measured 2 mm. in diameter.

Microscopic findings. The *standard mitral section* showed marked scarring of the valve. The vegetation noted grossly contained much calcium beneath which was a large amount of granulation tissue containing a variable number of polymorphonuclear neutrophils. Overlying the calcium was much fibrin containing many masses of bacteria and enmeshed intact red blood corpuscles. One area revealed alternating layers of partly organized fibrin and bacteria (Fig. 11). Numerous capillaries, some of them thrombosed, were present in the adjacent scarred valve. None was found to contain bacteria on examination of serial sections. The *standard tricuspid section* was taken to include the ulcer noted grossly. The valvar area, which grossly appeared ulcerated, revealed marked scarring and it was found that endocardium covered projections of scar tissue. No fibrin or bacteria were demonstrable.

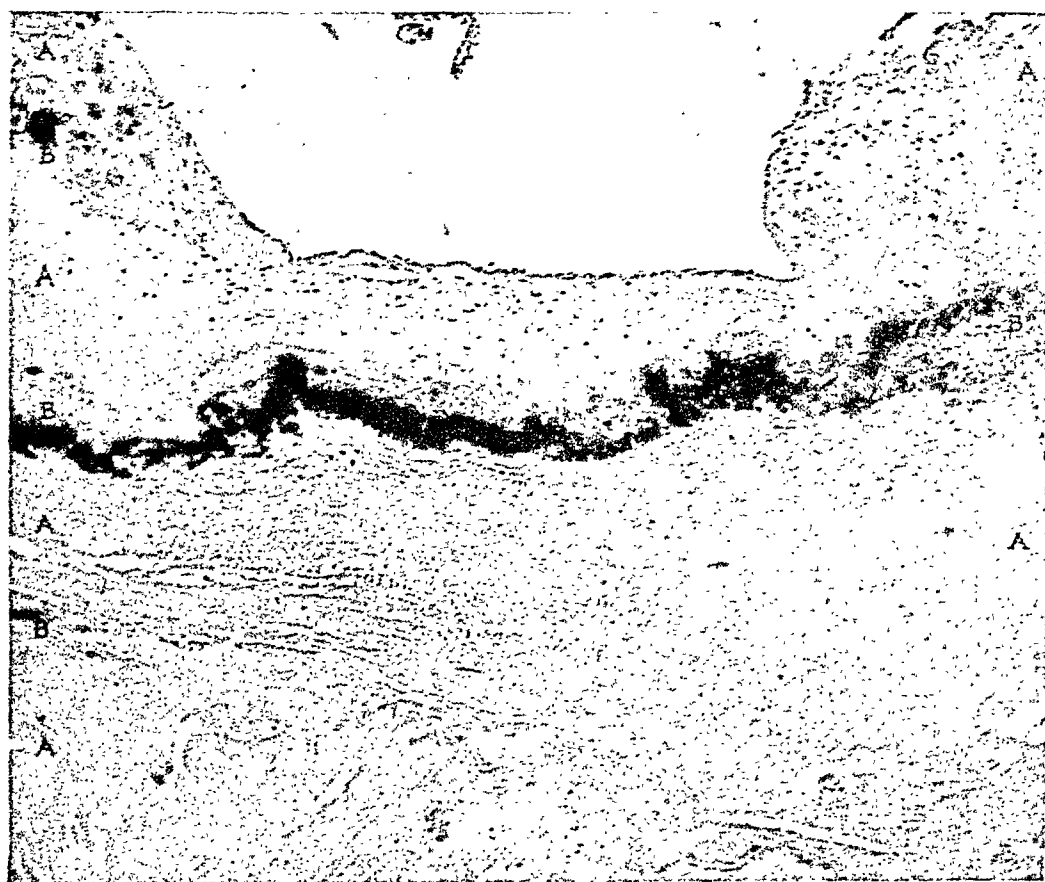
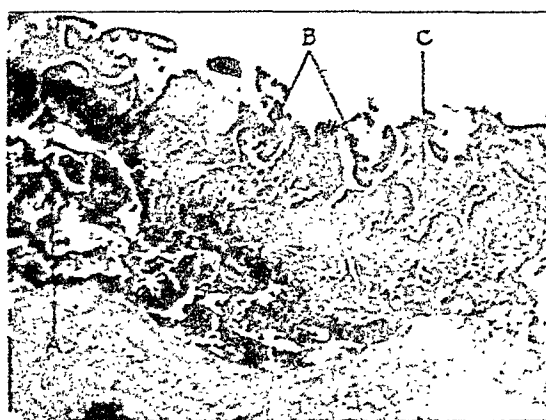
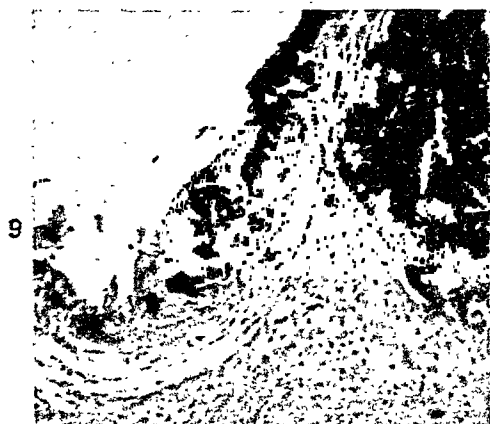
Case 5

Clinical data. The patient was a woman, aged 34, who gave no history of previous rheumatic fever and had been ill for approximately eight months prior to therapy. *Strepto-*

FIG. 9 (Case 3). Low power photomicrograph of valve lesion showing collections of bacteria covered by a layer of fibrin and platelets.

FIG. 10 (Case 6). Collections of bacteria on the free margin of the thin layer of fibrin covering remnant of the vegetation. Blood culture was negative prior to time of death. A, calcium; B, bacteria; C, fibrin.

FIG. 11 (Case 4). Low power photomicrograph showing layers of fibrin (A) in varying stages of organization with a zone of bacteria (B) near the surface and between each layer. The patient received anticoagulants throughout two courses of penicillin therapy.



coccus viridans was recovered from the blood; *in vitro*, its sensitivity to penicillin was 0.04 unit per cc. She had been treated elsewhere for many weeks, with daily doses of the drug, ranging from 100,000 to 200,000 units, without apparent effect upon the clinical course of the disease. She was given 400,000 units of penicillin daily for thirty days. Blood cultures became negative promptly after therapy was instituted and remained negative. The temperature, leukocyte count and sedimentation rate gradually became normal, but congestive heart failure developed on the twenty-fifth day and death ensued on the thirtieth day after the onset of treatment.

Gross findings. The heart weighed 320 gm. The left ventricle was slightly dilated; the myocardium was somewhat thinned, but otherwise apparently normal. The endocardium appeared normal except for the portion covering the mitral valve which showed several minute, brownish cream elevations, ranging from 2 to 7 mm. in diameter. The chordae tendineae were thickened, somewhat roughened, and five of them were broken (Fig. 4). Segments of the ruptured chordae tendineae extended from 2 to 7 mm. from the margin of the anterior leaflet. A roughened area was seen on the ventricular side of the cusp and an irregular minute patch of roughening was seen on the posterior wall of the left auricle.

Microscopic findings. Standard mitral sections revealed an intact endocardium over the scarred valve. The valve contained occasional capillaries and small masses of calcium. The adjacent myocardium disclosed no change except for slight diffuse fibrosis in scattered areas. In a section from the lesion of the mitral valve there was a defect in the endocardium in a very small area which was covered with a thin layer of fibrin, but no bacteria were seen. A few polymorphonuclear neutrophils and large mononuclear cells were found in this fibrin. Many fibroblasts were present in the underlying valve substance in which there was marked calcification (Fig. 7). In one portion, regenerating endocardial cells were seen overlying minute areas of organizing fibrin (Fig. 6). Sections of the left auricular wall revealed minute areas devoid of endocardium, beneath which were changes similar to those described above.

This is the only instance of all the patients who came to autopsy in which all the clinical and laboratory findings indicated recovery from the infectious process. It is, therefore interesting to note that endocardial regeneration was incomplete, and that the denuded surface was covered by a thin layer of fibrin.

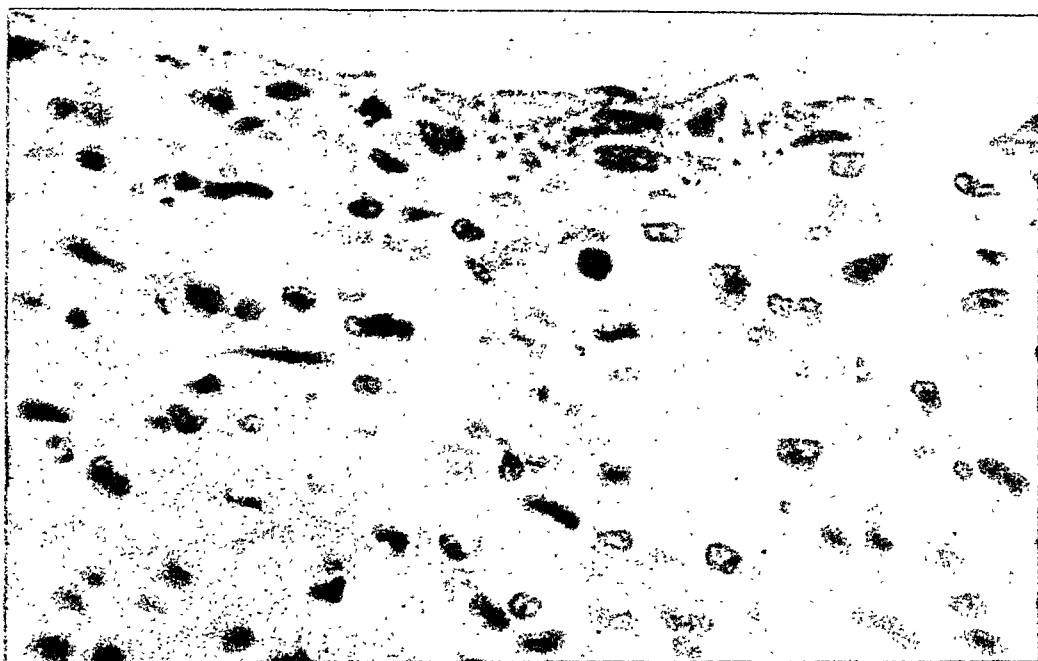
Case 6

Clinical data. The patient was a woman, aged 23, who gave a history of previous rheumatic fever and had been ill for approximately ten weeks prior to penicillin therapy. *Streptococcus viridans* was recovered from the blood; *in vitro*, its sensitivity to penicillin was 0.04 unit per cc. She was treated almost continuously with penicillin over a period of nine months, the dosage varying from 100,000 units to 2,000,000 units per day, but her blood cultures did not become permanently negative. During one course of therapy, lasting four weeks, 2,000,000 units per day were administered. Death occurred three days after starting a course of penicillin combined with Dicumarol and was due to massive cerebellar hemorrhage.

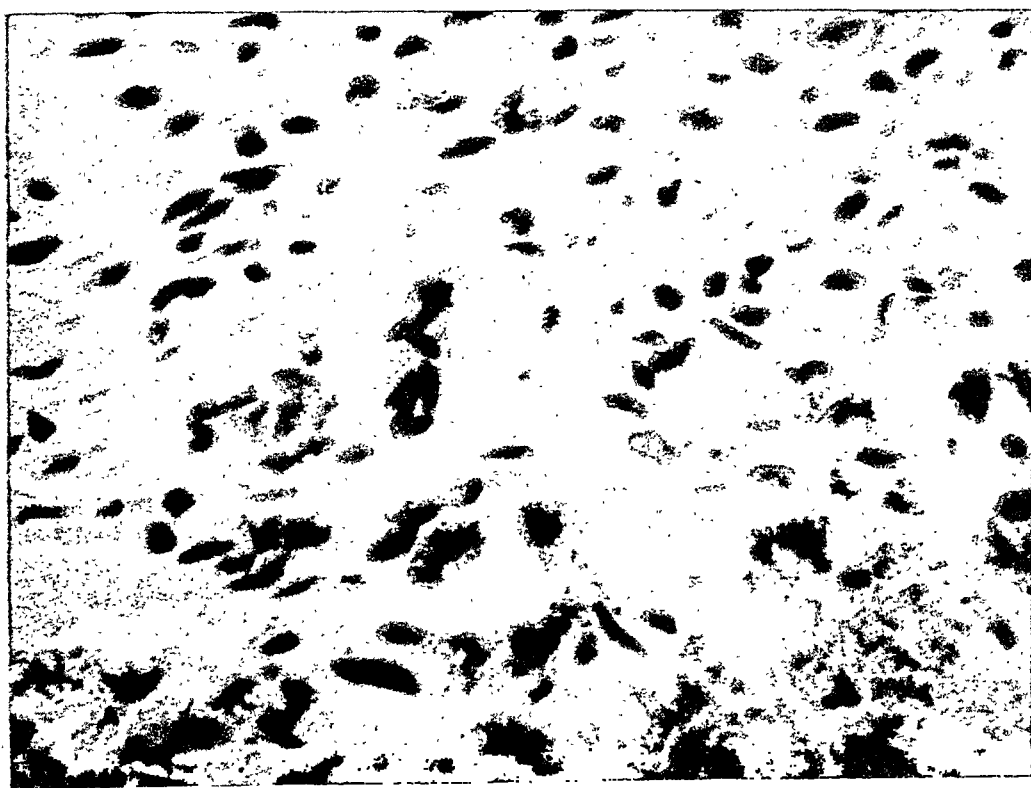
Gross findings. The heart weighed 210 gm. Two vegetations were found on the mitral valve. One of these was 5 mm. in diameter and light grayish tan in color and was found hanging by a thin thread of fibrous tissue 2 mm. in length. The other vegetation, at the edge of the valve, measured 4 mm. in length, was raised about 1 mm. above the surface, was irregular in shape and light pink in color. There was slight thickening and fibrosis of

FIG. 12 (Case 7). Bacteria invading endocardium.

FIG. 13 (Case 7). Multinucleated giant cells in the granulation tissue at the base of a healing vegetation.



12



13

the valve at the line of closure. The aortic, tricuspid and pulmonic valves showed no change.

Microscopic findings. The standard mitral section, taken through the larger vegetation noted grossly, revealed the lesion to occupy both surfaces of the valve edge and to be composed of fibrin containing many masses of bacteria and some calcium (Fig. 10). No evidence of inflammatory reaction was observed. The proximal portion of the valve was markedly scarred and contained an occasional capillary.

Case 7

Clinical data. The patient was a man, aged 69, who gave no history of previous rheumatic fever and had been ill for three months prior to penicillin therapy. While on our service, he received 500,000 units of penicillin daily for twenty-eight days. Cultures of the blood revealed *Streptococcus viridans* (*in vitro* its sensitivity to penicillin was 0.1 unit per cc.). Cultures became negative almost immediately after instituting treatment, and remained negative during treatment and during a subsequent period of twelve days of observation in the hospital. The patient appeared to have achieved a clinical cure. On the twelfth day after treatment was stopped, a routine blood culture showed *Staphylococcus aureus* which, at the time, was considered to be a contamination, but two cultures on the following day revealed the same organism. The patient had a severe chill, his temperature rose to 104 F.; he showed evidence of a right sided hemiplegia, and died a few hours later. *Staphylococcus aureus* was recovered from the heart's blood at autopsy.

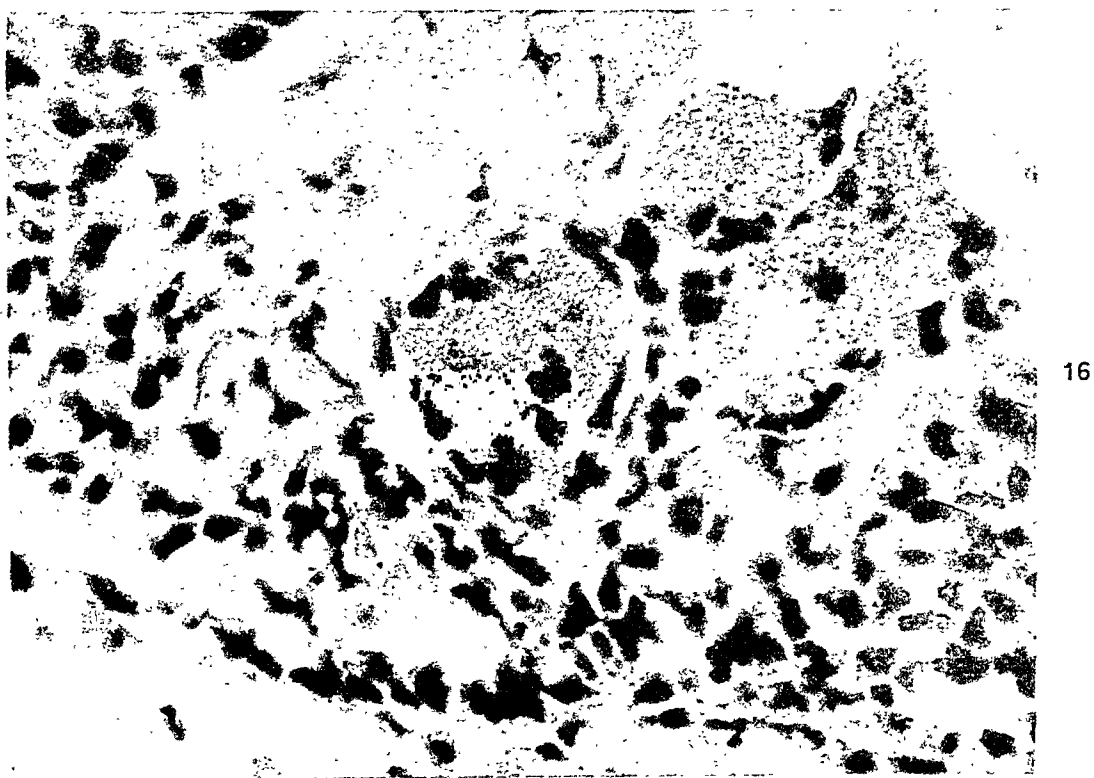
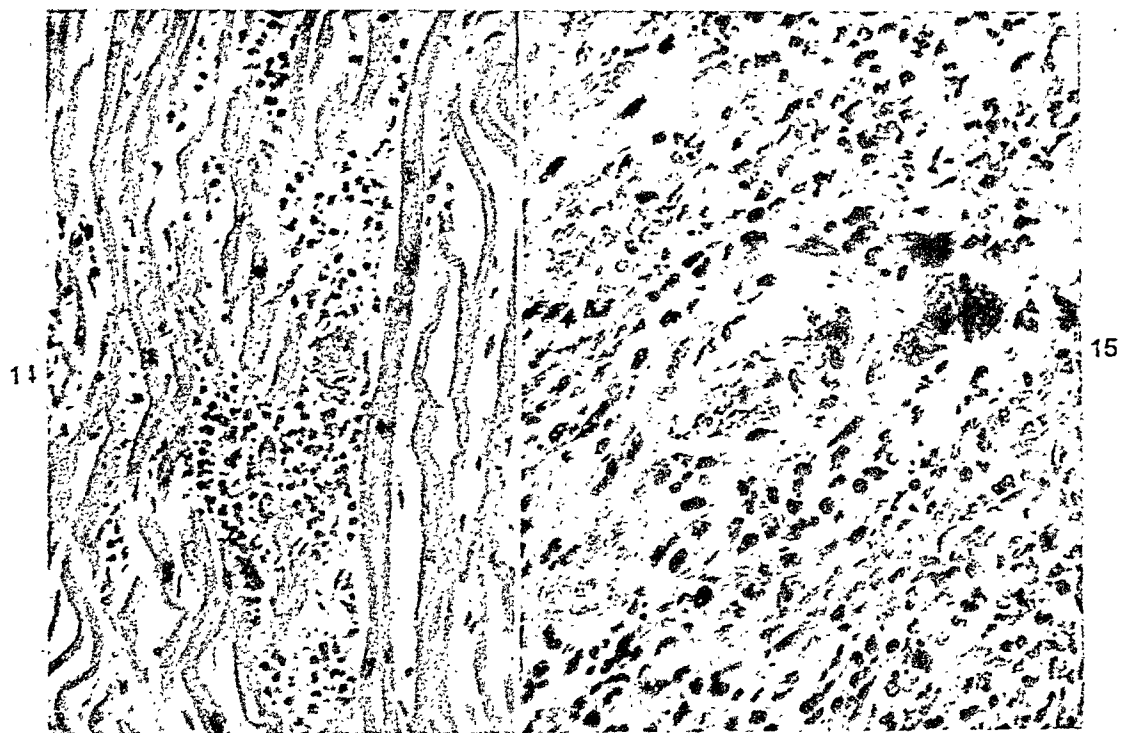
Gross findings. The aortic, pulmonic and tricuspid valves showed no change. The mitral valve was markedly thickened and several small, friable, slightly elevated red vegetations seemed to overlie scar tissue of the valve. The vegetations occurred on the auricular surface of the valve and occupied approximately half of the anterior cusp and a small amount of the medial portion of the posterior cusp. The chordae tendineae revealed thickening, scarring and shortening.

Microscopic findings. A section of the mitral valve revealed marked scarring. There were many apparently new capillaries and fibroblasts which seemed to run parallel with the surface. One of these capillaries contained masses of bacteria (Fig. 15). The midportion of the section showed ulceration of the endocardium with many superficial clumps of bacteria and an underlying mass of fibrin and red cells. The base of the ulcerated area contained many polymorphonuclear leukocytes and a large number of mononuclear cells which seemed to be embedded in the fibrin. Many fibroblasts and capillaries were also noted. The capillaries near the surface of the valve were filled, in part, with fibrin and, in part, with a mixture of clumps of polymorphonuclear leukocytes and red cells. These fibrin-filled capillaries were found on serial section to be continuations of more proximal capillaries filled with intact red blood cells. Many lymphocytes, large mononuclear cells and a few polymorphonuclear neutrophils surrounded the dilated capillaries. A clump of approximately 100 staphylococci was found deep within the scar tissue of the base of the ulcerated area. Serial sections revealed the bacteria to be within a capillary and to be extending into the adjacent scar tissue. No evidence of inflammatory reaction or of granulation tissue in the region of this capillary was noted. In additional sections, several endocardial cells revealed evidence of degeneration with partial fragmentation of the

FIG. 14 (Case 4). Focus of acute myocarditis.

FIG. 15 (Case 7). Low power photomicrograph of section from near the midportion of the mitral valve showing bacteria within a capillary with invasion of the surrounding tissue by bacteria. Bacteria were demonstrated to be cocci in clumps, but no chains were seen and blood culture at the time of death revealed *Staphylococcus aureus*. The patient had apparently recovered from a *Streptococcus viridans* endocarditis.

FIG. 16 (Case 1). Bacteria-filled capillary at the base of a vegetation. At the margin of the vegetation are seen small collections of fibrin with enmeshed cocci in short chains. Blood cultures were negative prior to death. Surrounding the capillary are masses of polymorphonuclear neutrophils, a few mononuclear cells and an occasional large phagocytic cell.



cytoplasm and pyknosis of the nuclei. These degenerating cells contained cocci varying in number from 2 to 20. Bacteria were found to extend a short distance beneath the endocardium in the involved area (Fig. 12).

Case 8

Clinical data. The patient was a man, aged 49, who gave a history of previous rheumatic fever and had been ill for approximately five months prior to penicillin therapy. *Streptococcus viridans*, having a sensitivity to penicillin *in vitro* of 6.0 units per cc., was recovered from the blood. He had received 300,000 units of penicillin daily for one month at another hospital. While under our care, penicillin was given for twenty days, in dosages up to 2,000,000 units daily. The total amount of penicillin administered was 39,355,000 units. Death was due to congestive heart failure with renal and cerebral complications.

Gross findings. The heart weighed 625 gm. There was marked dilatation of the left ventricle and slight dilatation of the right. The mitral valve was smooth and free from thrombi or vegetations. On the aortic valve there were three groups of firm, red, irregular vegetations, one group being present on the central portion of the free edge of each cusp. The largest vegetation measured 1.7 cm. in width and projected into the cavity of the ventricle for an extent of about 1 cm. There appeared to be some scarring beneath the vegetations. The tricuspid and pulmonic valves showed no change.

Microscopic findings. The standard mitral section revealed marked scarring and small amounts of finely divided calcium. Section of the right posterior cusp of the aortic valve showed slight scarring with marked thickening of the distal one-fourth. It contained many masses of calcium. The endocardium was defective and was replaced by fibrin containing many polymorphonuclear neutrophils. The fibrin seemed to extend from the end of the valve in a finger-like process containing three masses of calcium and small clumps of bacteria at the periphery. Overlying the fibrin was a thin layer of polymorphonuclear neutrophilic infiltration, which, in turn, was covered by a slightly thicker layer of red blood cells and scattered leukocytes loosely enmeshed in fine fibrin. Two true vegetations were noted in a small branch of the pulmonary artery. These were both essentially similar to that described above, containing fibrin, polymorphonuclear neutrophils and numerous, scattered, minute masses of blood pigment. However, no bacteria were found (Fig. 8).

Case 9

Clinical data. The patient was a man, aged 36, who gave a history of previous rheumatic fever and had been ill for approximately two and one-half months before coming under our care. He had been previously treated with 200,000 units of penicillin daily for three weeks and was discharged as cured. He was readmitted four weeks later with typical clinical findings of subacute bacterial endocarditis. Cultures of blood revealed atypical *Streptococcus viridans* which, *in vitro*, showed a sensitivity to penicillin of 0.8 unit per cc. Penicillin was administered continually for four and one-half months in dosages of from 500,000 to 2,000,000 units daily, but failed to produce persistently negative blood cultures. Death occurred from congestive heart failure. During the five days prior to death, the patient received 0.5 gm. of streptomycin daily.¹² *In vitro*, the sensitivity of the organism to streptomycin was 1.0 microgram per cc.

Gross findings. The heart weighed 650 gm. The external surface was covered with a thick layer of fibrin, beneath which were multiple petechial hemorrhages, especially numerous at the base where they coalesced into an ecchymotic area 2 by 2 cm. in size (Fig. 1). Both ventricles were moderately dilated and their musculature was slightly reduced in consistency. The aortic valve ring was fixed, scarred and calcific (Fig. 3). The opening was reduced to a slit measuring 1.5 cm. in length by 0.3 cm. in its greatest width, and was

partly occluded by firm verrucae and masses of calcific material, the latter apparently covered by endocardium. The ring measured 6 cm. in circumference. Two of the cusps (anterior and right posterior) were almost completely destroyed and replaced by large, irregular, fibrinous and fibrous, firm, well attached polypoid masses measuring 4 cm. in length and protruding from the surface for an average distance of 0.7 cm. There was an ulceration, 12 by 7 mm., on the ventricular surface at the base of the anterior cusp which extended through the endocardium to a maximum depth of 0.5 cm. The left posterior cusp was markedly scarred, thickened and calcified, most markedly at its free edge. At its junction with the anterior cusp, it merged with the above mentioned ulcerated area. There was a calcified plaquelike elevation 0.7 by 0.4 by 0.3 cm. in size on the commissure between the right and left posterior cusps. The mitral valve was markedly scarred, slightly calcified and inflexible. The opening measured 1 by 0.8 cm., and admitted the tip of the index finger; the ring measured 6 cm. in circumference. The valve was diffusely and markedly thickened. The greatly hypertrophied posterior papillary muscle was attached

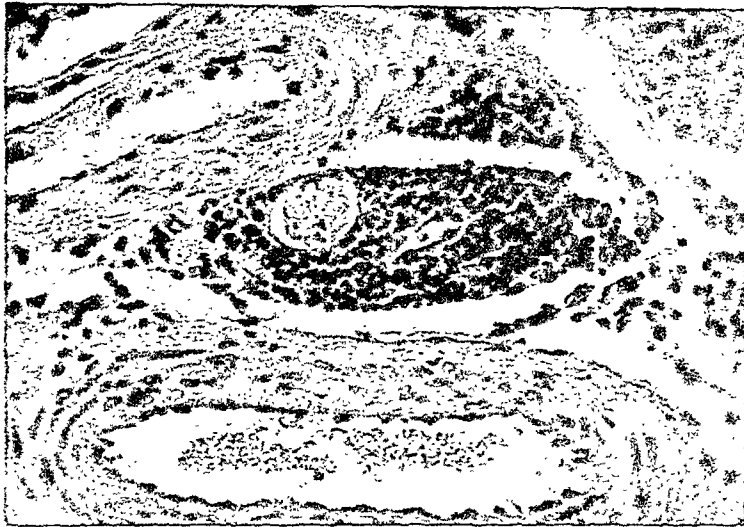


FIG. 17 (Case 1). Small myocardial abscesses adjacent to arteriole.

to the posterior leaflet by fused chordae tendineae, averaging 0.4 cm. in length and 0.5 cm. in diameter. On the auricular surface of the valve, opposite the midportion of the attachment of this papillary muscle, there was an ulcerated area measuring 10 by 3 mm., surrounded by scar tissue, and covered by multiple, extremely minute hemorrhagic elevations. The anterior papillary muscle was moderately hypertrophied and attached to its leaflet by moderately thickened, scarred and slightly shortened chordae tendineae.

Microscopic findings. The standard mitral section showed marked scarring and thickening. Much of the scar tissue was hyalinized and relatively acellular and contained small amounts of calcific material and numerous streaklike formations of unhyalinized fibrous tissue which seemed to accompany small blood vessels. In one area, scattered lymphocytes and polymorphonuclear leukocytes were found in this perivascular fibrous tissue. There were many dilated and congested blood vessels near the base of the valve, but no bacteria were found within these vessels. The endocardium overlying the valve was intact except in one area corresponding to the ulcerated area noted grossly. Here the endocardium was eroded. A small amount of fibrin and some intact red blood cells were present on the surface. A few large mononuclear cells and small accumulations of blood pigment were

seen immediately beneath this superficial layer. No bacteria were seen. It could not be determined with certainty whether the mitral valve and ring represented the healed stage of the bacterial endocarditis which was responsible for his first admission, or whether it represented the result of the previous rheumatic infection plus a beginning bacterial endocarditis. The adjacent myocardium revealed no change except for very slight diffuse increase in fibrous stroma. The endocardium overlying the myocardium was diffusely thickened and scarred. In the *standard tricuspid section*, the valve was slightly scarred and thickened, especially near the distal end. The endocardium appeared intact. Tissue from the ulcer of the *aortic valve* had to be decalcified prior to cutting the section and the cellular details were, therefore, somewhat obscured. The general contour of the valve was destroyed. It appeared to be formed of a mass of hyalinized fibrous tissue in which there were numerous accumulations of blood pigment in the unhyalinized perivascular fibrous tissue. The surface was considerably frayed; small amounts of fibrin were adherent to it and bacteria were not definitely identified. There was much evidence of calcification within the fibrous tissue, replacing the valve and even extending into the adjacent myocardium. Near the periphery of the myocardium were small accumulations of lymphocytes in the vicinity of blood vessels. These seemed to accompany small accumulations of blood pigment, most of which were present within macrophages. Otherwise, the myocardium was essentially similar to that previously described.

In a section from the *right ventricle*, the epicardial fat was normal in amount. The epicardium itself was largely replaced by a layer of fibrin and red blood cells in which no inflammatory cells or bacteria were found. There was much scattered recent hemorrhage into the epicardial fat while the myocardium was similar to that previously described. In a section from the *left ventricle*, the epicardial surface and fat were essentially similar to those described for the right ventricle. The underlying myocardium was also similar to that previously described, except that it contained many small dilated blood vessels.

DISCUSSION

The patients in Cases 1, 2, 3, 4 and 9 received anticoagulants along with part or all of their penicillin therapy. Details of our experience with the use of these agents has been reported in another paper.¹⁴

It was noted that within forty-eight or seventy-two hours after the start of penicillin therapy most of the subjective symptoms disappeared. The body temperature usually returned to normal and the blood culture became negative within the same period. The typical anemia tended to disappear gradually, even without blood transfusions or iron therapy. The frequency of appearance of petechiae was greatly diminished. Major embolic phenomena still occurred but less frequently than in untreated patients. In treated patients who recovered, however, progressively fewer episodes of embolism were noted. This sequence of events contrasts with the course of the disease prior to the advent of adequate penicillin therapy and represents clinical indication of healing of the endocardial lesions.

During the course of this study, it was learned that negative blood cultures might be misleading inasmuch as cultures could become negative under dosages of penicillin which were subsequently proved, both clinically and anatomically, to be inadequate for recovery. In other words, mere sterilization of the blood stream is not tantamount to cure. In all but one (Case 9) of our patients who came to autopsy, negative blood cultures were obtained repeatedly during treatment and, with one exception (Case 3), even up to the time of death, yet

in all but two patients (Cases 5 and 9) bacteria were found in the endocardial lesions. This apparent discrepancy suggests that the blood concentration of penicillin, although sufficient to sterilize the blood stream itself, was insufficient to inhibit growth of bacteria in the endocardial lesions.

At the onset of our studies, we postulated the obvious theory that in the presence of a fibrin barrier any antibacterial agent capable of inhibiting an organism *in vitro* must be capable of penetrating or diffusing through fibrin in a concentration at least equivalent to the sensitivity *in vitro* of the organism in question. The first failures with penicillin therapy meant either that penicillin could not penetrate fibrin or that the concentration after penetration of fibrin was inadequate. When larger daily doses of penicillin were used and blood levels considerably in excess of the sensitivity of the organism *in vitro* resulted in cures, clinical proof of the above theories was afforded. Nathanson and Liebhold¹² have furnished experimental confirmation of the ability of penicillin to penetrate fibrin. Likewise, it must be borne in mind that repeatedly negative blood cultures do not necessarily indicate continuous sterilization of the blood stream. Showers of bacteria undoubtedly do occur, as evidenced by the finding of bacteria in the capillaries of the valves at autopsy in spite of "negative" blood cultures taken just before death (Case 1). It is also interesting to note that in Case 1 repeatedly negative blood cultures were obtained during continuous intravenous administration of penicillin which was carried out during the day, but that a positive blood culture was obtained three hours after an intramuscular injection of penicillin administered during the night. Blood concentration of penicillin at this time was zero. This and other clinical observations led us to prefer continuous intravenous administration of penicillin in this disease.

All patients having an elevated or rising leukocyte count prior to the time of death were found to have active endocardial lesions, but normal leukocyte counts were present in some patients who showed active lesions, at autopsy. All patients with active endocardial lesions had persistently elevated or rising sedimentation rates prior to the time of death. Several of those who recovered had slight to moderate elevations of the sedimentation rate for from two to five weeks after completion of therapy. Examination of the pathologic material led us to the conclusion that elevation of the sedimentation rate in the absence of other sources of inflammation, indicates at least persistence of unorganized fibrin. As stated elsewhere,¹⁵ we believe that a steadily rising sedimentation rate during treatment is an indication for increased daily dosage and further therapy regardless of how favorable other signs may be, and, so long as the sedimentation rate remains elevated after therapy, the patient is vulnerable to reactivation and must be observed at very frequent intervals. If, during such observation following treatment, the sedimentation rate rises steadily, another course of treatment should be started, preferably with an increase in the daily dosage. Clinical experience as well as the histologic evidence here presented confirm, we believe, the wisdom of such a procedure. Scattered, minute areas of organizing bacteria-free fibrin may be present (Fig. 7) even though the sedimentation rate is normal.

Seven of the 10 patients who came to autopsy showed changes in serial electrocardiograms which were made during their illnesses. Acute myocarditis was present in the heart of each of these patients (Fig. 14). Such electrocardiographic changes have been noted in only 2 of 25 patients who recovered. Thus, serial electrocardiograms during therapy may aid in estimating the presence and severity of acute myocarditis.

Congestive heart failure developed late in the disease in those patients who, at autopsy, showed most marked evidence of myocardial damage, and in those showing the most extensive valvar lesions, *viz.*, severe ulceration, calcification and scarring of the valves, or rupture of the chordae tendineae (Figs. 3 and 4). Clinical evidence of congestive heart failure was rarely observed in patients who recovered.

According to the literature, 75 per cent of patients developing subacute bacterial endocarditis have previously had rheumatic heart disease. A positive or suggestive history of previous rheumatic fever could be obtained in less than 50 per cent of all our cases. The valves of all patients at autopsy, however, showed evidence of previous damage. It could not be determined with certainty in every case whether this damage was due to earlier infections or to changes attributable to the age of the patient.

Streptococcus viridans was the infecting organism in all but one of the cases described in this report, although among the recovered patients, *Streptococcus anhemolyticus* and *hemolyticus*, *Staphylococcus aureus* and *albus* and *H. parainfluenzae* were found. The sensitivity of the microorganism to penicillin *in vitro* was not, in every instance, a criterion of the dosage of penicillin required or of the response to penicillin therapy.¹⁵

Cardiac pathologic anatomy. In our cases, the valvar lesions were microscopically similar to those usually encountered prior to penicillin therapy. Grossly, they were much smaller, quite firm and appeared as fibrous tags or elevations covered by fibrin (Fig. 5). Polypoid fungating masses were not seen except in Case 2 (Fig. 2). Extension of the lesions to the chordae tendineae with possible rupture is described in Case 3 and Case 5 (Fig. 4).

Microscopically, the lesions in 7 of the 9 cases were composed of bacteria, fibrin and platelets, situated on a denuded endocardial surface (Fig. 10). Theoretically, in such instances the blood culture should be positive, but negative cultures were obtained immediately before death in 5 of these 7 cases. It might be assumed that these surface colonies of bacteria had been inhibited by the concentration of penicillin in the blood stream, which theoretically was more than adequate. While disintegration and poor staining qualities were occasionally observed, however, these phenomena were not sufficiently general in any lesion to indicate inhibition of all organisms. In all of these cases, bacteria covered by a layer of fibrin and platelets were also seen (Fig. 9). A more advanced stage of this process is illustrated by Figure 11 which shows thick layers of organized fibrin overlying zones of bacteria in the valve substance. The bases of the lesions revealed varying degrees of organization with formation of fibrous tissue. Organization was accompanied by calcification and in 4 cases calcifica-

tion was severe (Figs. 2, 4, 7, 10). New capillary formation was frequently seen. These evidences of healing were more marked in our cases than in those described in the literature prior to the use of penicillin. In the specimen showing virtually complete healing (Case 5), the fibroblastic invasion in the area of organization extended to the valve margin (Fig. 7). Many lymphocytes, large mononuclear cells and plasma cells were present. Multinucleated giant cells were also noted (Fig. 13), and appeared to be engaged in the removal of necrotic material. Polymorphonuclear neutrophils are generally described throughout the literature as being absent or being present only in small numbers, but Boyd¹ mentions that they may be present in large numbers. In our material, polymorphonuclear leukocytes were numerous in most of the sections studied and frequently were the predominating cell.

As far as we know, endocardial regeneration has not been previously described. This phenomenon was present in Case 5 (Fig. 6).

Reports concerning myocardial lesions in subacute bacterial endocarditis are conflicting. According to most observers, cloudy swelling and fatty degeneration of the muscle are the rule, but this was not true in our cases. Other observers comment on myocardial fibrosis and this change was present in most of our material. Clawson and Bell⁴ demonstrated Aschoff bodies in 11 per cent of their cases and Buchbinder and Saphir³ found them in 15 per cent of 40 cases. We found none. Although serial sections of the myocardium were not made, the areas we studied should, according to Gross,² show Aschoff bodies if present. One case revealed necrotic areas of myocardium with polymorphonuclear infiltration. Saphir¹⁶ believes that these are "myocardial abscesses." He also described fairly widespread acute myocarditis and myocardial infarction. De Navesquez⁷ described myocardial lesions in 19 of 20 cases examined by serial sections and considered them to be due to embolic phenomena. Fibrin-filled capillaries were found in many of our myocardial sections. Our finding of acute myocarditis (Fig. 4) in 7 cases and myocardial abscess in 1 case conforms with the experience of Saphir.

Pericardial inflammation is seldom seen unless active rheumatic heart disease occurs just prior to, or concomitantly with, subacute bacterial endocarditis.¹⁴ Acute fibrinous pericarditis was present in one of our cases (Fig. 1). Active rheumatic heart disease was not present at the time.

PATHOGENESIS OF SUBACUTE BACTERIAL ENDOCARDITIS

The actual mechanism by which the endocardium becomes involved in subacute bacterial endocarditis is not clearly understood. Bacteria may become implanted on unhealed rheumatic lesions or become implanted on minute platelet thrombi which occur on the endocardium overlying the damaged valve. Trauma of the valve at the points of closure, and bacterial embolism of valve capillaries have also been mentioned.^{5, 6, 8, 10, 17} In our material, Case 7 illustrates the implantation mechanism. Bacteria were demonstrated invading endocardial cells, in the absence of platelet thrombi, and extending into the subendocardial tissues (Fig. 2). Cases 1 and 7 illustrate invasion of the valves

by way of capillaries, presumably formed during previous infections (Figs. 15 and 16). Bacteria-filled capillaries were found in the midportion and near the margin of the valves. They were traced by serial sections and found to be continuations of more distal capillaries filled with blood. Case 4 revealed a valve capillary filled with fibrin which contained no bacteria. Case 7 represents a patient who, apparently cured of a viridans endocarditis and just prior to being discharged from the hospital, died of a staphylococcus septicemia seventy-two hours after onset of symptoms. The only unfavorable sign at the time the staphylococcus septicemia developed was a moderately elevated sedimentation rate. No evident portal of entry for the staphylococcus was found. At autopsy, the involved valve still showed the presence of fibrin. Enmeshed in the fibrin were myriads of cocci, none of which appeared to be in chains. Likewise, none of the bacteria in the capillaries showed chain formation. That these capillaries antedated the staphylococcus septicemia, there can be no doubt. Owing to the short time elapsing between the initial chill and death, it seems highly probable that direct invasion of the previously damaged endocardium and invasion of the valves *via* the capillaries took place. Based on studies of the other hearts, we believe that at least some of the fibrin in this case was a residual of the viridans endocarditis and formed an excellent site of implantation and probable culture medium, for the organisms of a transitory staphylococcus bacteremia. In addition to illustrating the two modes of valvar invasion, this is extremely significant evidence, based on pathologic findings, that following apparent recovery from subacute bacterial endocarditis the involved valve is still vulnerable to reinfection for some time. This vulnerability to reinfection seems to last as long as the sedimentation rate remains elevated. Following apparent clinical recovery, it would appear desirable to administer moderate doses of penicillin so long as the sedimentation rate is still elevated in order to combat transitory bacteremias.

SUMMARY

In 34 cases of subacute bacterial endocarditis receiving penicillin therapy, early subjective improvement was noted in every patient regardless of the ultimate outcome. In spite of repeatedly negative blood cultures, at autopsy, bacteria were demonstrated in the valvar lesions in 7 of 9 cases.

Elevated or rising leukocyte count prior to the time of death was found to coincide with the presence of active endocardial lesions. Active lesions were also found associated with a normal leukocyte count. Changes in serial electrocardiograms were found to coincide with histologic evidence of acute myocarditis. All cases with elevated or rising sedimentation rate at the time of death showed active valvar lesions. In one case, with a normal sedimentation rate, microscopic areas of bacteria-free organizing fibrin were present.

The majority of the endocardial lesions in these patients treated with penicillin were small and firm and showed marked evidences of healing. Calcification was, at times, of such severe degree as to be an important factor in inducing fatal congestive failure. Deposits of finely divided calcium were frequently

found in the uninvolved valves. The completely healed valve is thickened and scarred and grossly resembles the healed stage of rheumatic valvulitis. Polymorphonuclear leukocytes were more frequently noted than has previously been described in the literature. Fibrin was found to persist at the site of endocardial lesions after clinical "recovery" had occurred. It is postulated that this fibrin serves as an excellent implantation site and culture medium. Reinfection with the same, or with other bacteria, thus may occur unless adequate penicillin therapy is maintained for a sufficient length of time to allow the endocardium to regenerate. Endothelial regeneration was demonstrated histologically in one case.

Extensive myocardial lesions were found in all autopsies. True vegetations in a small branch of the pulmonary artery were seen in one case (Fig. 8). So far as we know, this is the first time such lesions have been described in the smaller arteries.

Histologic evidence is presented that infection of the cardiac valves in the genesis of subacute bacterial endocarditis may occur by direct endocardial invasion without the presence of platelet thrombi and *via* capillaries resulting from a previous inflammatory process. Both mechanisms may operate simultaneously.

No increase in resistance of the organism to penicillin *in vitro* was encountered in 7 patients who received more than one course of treatment. This was also true in those patients who, after one or more failures, ultimately recovered with administration of the drug in adequate daily dosage.

CONCLUSIONS

In patients treated with penicillin, the lesions of subacute bacterial endocarditis revealed marked evidence of healing.

The degree of healing appeared to be definitely related to adequacy of daily dosage and duration of treatment. Adequacy of daily dosage cannot be judged by mere sterilization of the blood stream. Inadequate daily dosage over a long period resulted in almost complete healing in 8 cases, but with persistence of bacteria-laden fibrin in 7. Our experiences in these cases, as well as our experiences with the patients who recovered, have led us to adopt 500,000 units per day as the minimal dose.

The healing process is the same as that presented in standard textbooks of pathology except that the process is more advanced in patients treated with penicillin and acute inflammatory reaction is much more marked than is described in the medical literature to date.

Even in the presence of clinical and bacteriologic "cure," thin layers of fibrin may remain on the endocardial surface for several weeks.

Highly suggestive evidence is presented that infection or reinfection of the valvar endocardium can occur either by direct bacterial invasion of the endocardial cells without the presence of platelet thrombi, or *via* the subendocardial capillaries.

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USE OF PAPAIN IN CULTIVATION OF BACTERIA FROM THE BLOOD*

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A method for the cultivation of bacteria from blood has to fulfill a number of basic requirements: avoidance of contaminations, use of highly sensitive culture mediums, neutralization or decrease of the bactericidal power of the blood and of the bacteriostatic action of drugs and addition of an anticoagulant. Since we were impressed, on the one hand, with a wide divergence of opinion regarding the value of active trypsin preparations and, on the other hand, with the favorable results reported with the use of these preparations, especially for primary isolation of micro-aerophilic organisms from the blood,^{2, 9, 14} we were led to investigate the value of another proteolytic enzyme, papain, in blood cultures. It was believed that this enzyme might have certain advantages over trypsin. It has a wider, and possibly more suitable range of activity, its limits of pH varying from 5 to 7. Furthermore, it is cheap and easily obtainable.

The ability of papain, when activated, to interfere with the action of antibodies on bacteria has been proved by Kalmanson and Bronfenbrenner.⁷ Papain was used instead of trypsin in "papain digest broth" by Ungar.¹² This medium does not seem to be identical with the one we propose, since it apparently belongs to the "digest" type of medium. When this enzyme is used in chemical processes, an activator is usually employed (cysteine or hydrocyanic acid). Ungar's investigation seemed to bear out the belief that the inclusion of an activator, undesirable as this was for bacteriologic reasons, was superfluous for our purpose.

In the present study, a preparation obtained from local *Carica papaya*† was used in concentration of 2 per cent of the powder suspended in normal saline. Once a fortnight this suspension was filtered through paper after twenty-four hours' standing, then passed through a Seitz filter and kept in the refrigerator. It was a clear, yellowish fluid, which had pH 6.4 (potentiometer) and the following chemical content: total protein, 1.23 per cent; glucose, 31 mg. per 100 cc. (Folin-Wu); residual nitrogen, 66 mg. per 100 cc.; cysteine; and nicotinic acid, 22 gamma per gram of dry substance.‡ Its enzyme activity was borne out, for instance, by fibrinolysis of clotted blood added to it and, as I observed at the beginning of the work, by an immediate anticoagulant effect when fresh blood in an amount of about 5 cc. was added to not less than 3 cc. of papain-saline. It was observed that smaller amounts of papain-saline did not prevent coagula-

* Received for publication, January 6, 1947.

† I wish to acknowledge, with thanks, the supply of this material prepared according to the British Pharmacopoeal Codex by "Zori," Tel-Aviv Pharmaceutical and Chemical Manufacturing Products Company.

‡ Unpublished microbiologic method (Dr. N. Grossowicz).

tion of the blood although visible fibrinolysis started after fifteen to thirty minutes. To papain has been ascribed a multiple effect on blood coagulation.^{3,5} I have observed its accelerating action on clot formation in oxalated plasma, and its anticoagulant and fibrinolytic effect on blood.

In order to evaluate the suitability of this preparation for blood culture work, it was necessary to investigate its promotion of growth of certain bacteria, as well as its neutralization of inhibiting effect of a sulfonamide or of penicillin. Secondly, a comparison of blood cultures examined by a standard method and by a method using papain-saline had to be carried out.

Papain-saline was added to plain broth or brain heart infusion in a 10 per cent concentration. Preliminary experiments showed that the addition of papain to broth did not inhibit the growth of bacteria and, in some instances (*Staphylococcus aureus*, *E. typhosa*, streptococci), even seemed to stimulate it. It was soon noticed that, even in papain-saline without broth, staphylococci, various streptococci and laboratory strains of *Brucella melitensis* and *E. typhosa* were able to grow. The growth-promoting effect was especially evident for streptococci, particularly those of the viridans group. Several strains were encountered which, as might be expected, could not be cultured in broth or brain heart infusion, and not even when serum was added, although growth readily occurred on primary isolation in papain-saline broth or papain-saline brain heart infusion. A micro-aerophilic strain of *Clostridium butyricum* could be cultivated in papain-saline brain heart infusion, whereas it did not grow in brain heart infusion; *Cl. botulinum* grew in neither. The results regarding *H. influenzae* are still inconclusive.

As experience in blood cultures showed that the number of cultures positive for *Staphylococcus albus* was less in papain-saline than in the ordinary cultures, the growth of several strains of *S. albus* was tested in brain heart infusion and papain-saline brain heart infusion and papain-saline broth. While these strains provided a rich growth in the accepted mediums, they grew poorly and only within a comparatively small area beneath the surface in papain medium. As compared with ordinary mediums, the presence of papain apparently improves growth conditions for micro-aerophilic organisms, or at all events slightly reduces oxygen tension. This hypothesis is based on the reaction of certain streptococci and *Cl. butyricum* mentioned above. Possibly, the prevailing oxygen tension is unfavorable to *Staphylococcus albus*.

As a preparation containing plant protein might possibly contain para-aminobenzoic acid, tests were made in order to discover the sulfonamide-inhibiting properties of papain-saline. Serial 1:2 dilutions of sodium sulfathiazole in 1 cc. of broth, brain heart infusion and papain-saline broth, respectively, were inoculated with 0.1 cc. of 1:100,000 dilution of twenty-four hour cultures of different strains of *Staphylococcus aureus*. Brain heart infusion had a more pronounced sulfonamide-inhibiting effect than plain broth; but the addition of 0.05 cc. papain-saline to broth evidently neutralized a much larger proportion of the sulfonamide, as is shown in Table 1.

Since a preliminary series of experiments, in which penicillin, in amounts of 8

to 16 units, was dissolved in 2 cc. papain-saline (four hours, 37 C.), failed to clarify the question of whether the papain preparation was capable of neutralizing penicillin, a second series of experiments was set up, in which penicillin, in amounts of only 4 to 8 units, was incubated in papain-saline and normal saline, respectively, for forty-eight hours. A third determination of penicillin content was performed simultaneously with a freshly prepared penicillin dilution. Twenty-four hour cultures of *Staphylococcus aureus* (0.1 cc. of 1:100,000 dilution,)

TABLE 1

EXTENT OF GROWTH* OF 0.1 CC. OF 1:100,000 DILUTION OF A 24 HOUR CULTURE OF *Staphylococcus aureus* 309 IN SEVERAL MEDIA CONTAINING VARIOUS AMOUNTS OF SODIUM SULFATHIAZOLE

SODIUM SULFATHIAZOLE	BROTH 1.0 CC.	BRAIN HEART INFUSION 1.0 CC.	BROTH 1.0 CC. AND PAPAIN-SALINE, 0.05 CC.
375 mg. per 100 cc.	0	+	++
250	±	+	++
125	±	++	+++
60	+	++	+++
30	++	+++	++++
Control	+++	++++	++++

* 0 = no growth, + = slight growth, ++ = moderate growth, +++ = heavy growth and ++++ = very heavy growth.

TABLE 2

COMPARISON OF THREE PENICILLIN-CONTAINING MEDIA, INDICATING MINIMUM AMOUNTS OF PENICILLIN NECESSARY TO ALLOW GROWTH OF A STRAIN OF *Streptococcus viridans* AND A STRAIN OF *Staphylococcus aureus**

ORGANISM	RESISTANCE OF ORGANISM IN TERMS OF INTERNATIONAL UNITS OF PENICILLIN PER CC. IN MEDIUM CONTAINING:		
	Saline, incubated 37 C., 24 hr.	Papain incubated 37 C., 24 hr.	Penicillin freshly diluted in saline
<i>Streptococcus viridans</i> 2330P . . .	0.12±	0.5++	0.03+
<i>Staphylococcus aureus</i> 2526	0.015+	0.12+	less than 0.006

* ± = trace, + = slight growth, and ++ = moderate growth.

or streptococci (0.1 cc. of 1:10 dilution,) were used. The result in seven experiments showed a difference of from 1 to 3 tubes between the series of saline penicillin and papain-saline penicillin dilution, and a difference of 1 tube between saline incubated and freshly diluted penicillin, as may be seen in Table 2.

This decrease in penicillin activity observed after incubation with papain-saline is likely to be of practical importance in blood culture specimens. The facts observed find their explanation in the work of Lawrence⁵ who stated that many enzyme preparations, such as takadiastase and clarase contain small quantities of penicillinase, independent of their enzyme concentration. He conclusively proved that this penicillinase is the product of bacteria contaminat-

ing the powdered enzyme preparations. Pure papain has been proved by Lawrence⁵ to be inactive towards penicillin. Following his experiments, I isolated the bacterium present in my preparation and ascertained the penicillinase activity in broth after it was passed through a Seitz filter, and determined that 0.05 cc. of this filtrate was able to inactivate at least 8 units of penicillin in three hours (37 C.). This organism apparently belonged to the group of *B. megatherium*. It was a long, gram-positive, spore-forming bacterium which grew easily on agar and in broth.

Finally, papain-saline proved to be anticomplementary. When examined with the hemolytic system of the Wassermann reaction, it completely bound complement in concentrations of 0.2 or more.

MATERIAL

The specimens taken from the Medical and Pediatric Departments of our hospital included a rather large number of cases of septicemia and allied conditions from which repeated cultures were selected for our work. This fact largely explains the high percentage of positive findings.

METHOD

Ten cc. of venous blood (for the papain cultures, for technical reasons, sometimes less than 10 cc.) were placed into each of two Erlenmeyer flasks, one containing 3 cc. of 2.5 per cent of sodium citrate, the other 5 cc. of papain-saline. From each flask, two plates were poured, using 1 cc. blood for each plate and 2 per cent glucose agar. When anaerobic organisms were suspected, another 1 cc. was transferred to Tarozzi medium. When brucellae were suspected, cultures were taken and handled separately. To the remaining blood, 50 cc. of brain heart infusion (Difco) was added to each flask. The cultures were incubated at 37 C. for seven days and were examined daily with Gram stain and subcultured on rabbit blood agar and other mediums, when so required. When a positive result was obtained from one flask, the other was kept for control up to seven days. Para-aminobenzoic acid was added to specimens containing sulfonamides, but later it was added only to the cultures without papain-saline.

Altogether 132 double cultures were taken, and another 13 only with the papain method. These latter cultures are included in the evaluation of the new medium, and for the purpose of comparison the results of both series are corrected by statistical methods.

RESULTS

Table 3 illustrates the findings recorded according to the two methods employed and the different organisms isolated.

A fair number of the contaminations were encountered during a short period for which there was a rather instructive explanation. The disinfectant in which sterile forceps were kept in the wards, at that time, contained *Pseudomonas fluorescens* and this organism promptly appeared in our cultures.

TABLE 3
SUMMARY OF CULTURE MEDIUMS USED AND OF ORGANISMS ISOLATED,
ACCORDING TO MEDIUM USED

Culture	Number
Double cultures	132
Papain cultures only	13
Total number of papain cultures	145

(Number of patients examined

67)

Results

ORGANISM	PAPAIN CULTURES			DOUBLE VALUE OF STANDARD ERROR OF DIFFERENCE*
	Double cultures		Papain brain heart infusion only	
	Brain heart infusion	Papain brain heart infusion		
<i>Staphylococcus aureus</i>	21	25	3	9.12
<i>Staphylococcus albus</i>	35	22	3	9.9
<i>Staphylococcus citreus</i>	—	1		
<i>Pneumococcus</i>	2	2		
<i>Streptococcus haemolyticus</i> ...	—	1	1	
<i>Streptococcus viridans</i>	4	13		
	(in 18 glucose- agar plates: 9)†	(in 17 plates: 7)†		6.32
Diphtheroids.....	6	5		
<i>B. proteus</i>	—	1		
<i>E. typhosa</i>			1	
Sarcine.....	—	1		
Contaminants.....	21	31	1	9.6

* When the difference between the values of both examinations exceeds double the standard error of difference, this difference is unlikely to be due to mere chance and may, therefore, be considered significant.⁵ The percentages have been figured out in the case of papain cultures, by considering the total number of cultures taken by this method and the total results. The formula used to obtain the standard error of difference (S.E.D.) was as follows:

$$\text{S.E.D.} = \sqrt{\frac{p_1 \times q_1}{n_1} + \frac{p_2 \times q_2}{n_2}}$$

where

p_1, p_2 , = respective percentages of positives

q_1, q_2 , = respective percentages of negatives

n_1, n_2 , = respective numbers of observations.

1 refers to the series of brain heart infusion cultures; 2 refers to the series of papain brain heart infusion cultures.

† In 18 glucose agar plates poured from 9 specimens of blood, 9 were positive for *S. viridans*, and in 17 glucose agar plates poured from 9 specimens of blood, 7 were positive for *S. viridans*.

Several times, growth was obtained twenty-four to forty-eight hours earlier with the papain method. On at least four occasions, positive results were obtained only in the papain-saline cultures when the patients were undergoing

penicillin treatment. As to the glucose agar plates, in no instance were bacteria isolated by this method alone.

DISCUSSION

Taking into account the sensitive medium used in the controls and the large number of specimens tested repeatedly from cases of septicemia, including five cases of subacute bacterial endocarditis, a considerable difference in positive findings could not be expected from any method under investigation. In spite of this we feel justified in pointing out certain advantages of the papain method. While the number of significant findings is at least equal to that of our routine cultures, the number of isolations of *Staphylococcus aureus* is somewhat larger than in the controls. Streptococci of the alpha hemolytic type have been isolated definitely more often than by the citrate method and positive results were obtained earlier on several occasions. The smaller number of findings of *Staph. albus* does not appear to be a real drawback. There is no reason, according to our experience, to assume that a rare case of *Staph. albus* bacteremia would evade detection by the papain method. This point is borne out by two cases of *Staphylococcus albus* septicemia in which this organism was repeatedly isolated with both methods.

Some of the anticoagulants in common use, such as sodium citrate^{1, 4, 14} and liquoid,^{11, 13} have definite growth-inhibiting qualities. Sodium citrate is not a nutrient substance for most bacteria while saponin, so useful for the isolation of *Streptococcus viridans*, has been proved inferior to glucose trypsin broth for recovery of *Staphylococcus aureus*.¹⁰ A comparison between papain and trypsin made above, points to certain advantages of the former. Trypsin, moreover, is not known to exercise an antagonistic effect on penicillin and sulfonamides.

Papain-saline provides a culture medium from the very moment the blood is drawn into the flask. At the same time, it serves as an anticoagulant, an inhibitor of penicillin and sulfonamides and as a complement-fixing substance, qualities which when taken together, to the best of my knowledge, have not been claimed for any single preparation. It possibly digests some of the proteins of the blood (personal observations) and of the nutrient medium, thereby replacing the weak enzyme apparatus of fastidious bacteria. In view of the fact that this preparation is at least equivalent to an accepted standard method, that it is definitely superior for the isolation of streptococci and also enables the growth of micro-aerophilic organisms, it seems that the number of bacteria whose cultivation may be expected in this medium would be rather large. Owing to its fibrinolytic action, papain might also be used when clot cultures are to be made. Its use should be indicated, according to my experience, particularly in subacute bacterial endocarditis.

SUMMARY

A sterile preparation of 2 per cent papain powder (B.P.C.) in normal saline solution was examined for qualifications as a blood culture medium together with a fluid medium. Papain-saline proved to be a nutrient medium itself. Some

observations were made on its influence on the growth of several species of bacteria. A definite enhancement of growth was noted for certain bacteria, particularly streptococci.

Papain-saline was found to be an anticoagulant when employed in adequate amounts, to counteract the effect of certain sulfonamides and, to some extent, the effect of penicillin *in vitro*, and to bind complement.

An attempt was made to determine the value of papain-saline when employed in blood cultures. A total of 145 cultures containing papain-saline were examined, 132 of them with simultaneous controls. The papain method gave results which were at least equal to the controls, with the possible exception of *Staphylococcus albus*. It proved to be superior for the isolation of streptococci of the viridans group (13 positive findings as compared with 4 in the controls). Certain other advantages may be expected from its use in a blood culture medium.

Acknowledgments. I wish to express my deepest gratitude to Dr. Josef Gurevitch, Head of the Bacteriological and Serological Laboratory of Hadassah, without whose kind help and advice this investigation would not have been possible; to the Hadassah Medical Organization and to Doz. Dr. J. Kleeberg, Head of Medical Department A. for the special arrangements made to enable me to carry out this work; Dr. N. Grossowicz for valuable advice; Miss J. Benderski for the chemical analyses; and the staff of the bacteriologic laboratory for its kind cooperation.

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SPONTANEOUS RUPTURE OF SPLENIC ARTERIAL ANEURYSMS.

REPORT OF THREE CASES*

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Because of their rarity, it is desirable to report cases of splenic arterial aneurysms, particularly those which rupture spontaneously and those which occur as complications of pregnancy. Approximately 144 cases have been reported. Our report includes a review of 107 of these together with the records of three of our own cases.

Sherlock and Learmonth⁴⁰ found 0.039 per cent of splenic arterial aneurysms in over 84,000 autopsies from collected reviews. In the 98 cases in which the sex of the patient was known, there were 60 females and 38 males. The average age was about 48 years in each of the two sexes. In the younger patients, there was a higher percentage of females, but in the older patients the sexes were almost equally represented.

OCCURRENCE OF SPLENIC ANEURYSM IN PREGNANCY

Of the 60 females in this series, 15 were pregnant. These cases are summarized in Table 1. The rupture was fatal in 14 of these 15 cases. In 8, the lesion was considered to be congenital, while 1 was classed as mycotic secondary to a rheumatic valvulitis. Parity was not mentioned in a sufficient number of the cases to be significant. Only 1 of the 15 had any evidence of pregnancy. In 12, there was a rather sudden onset of intra-abdominal hemorrhage without premonitory symptoms.

PATHOGENESIS

In 84 cases the number and location of the aneurysm was mentioned. In 62, the aneurysm was single and in 22 multiple, while in one patient, there were as many as ten small aneurysms. The main splenic artery was involved somewhat more frequently than were the branches. The average diameter of the aneurysms was 2.3 cm. In 63 cases there was sufficient gross description to judge the site into which rupture of the aneurysm had occurred. The area involved and frequency are indicated in Table 2.

Other gross features included varying amounts of pancreatic destruction and calcification, thrombosis of the splenic artery and vein and ischemia of the abdominal organs. In most instances the spleen was enlarged and very often was infarcted. The pathogenesis in 63 cases is summarized in Table 3.

In arteriosclerotic individuals the aneurysms were found usually along the main artery. The average age of these patients was 60 years. There was an approximately equal number of patients of each sex. The lesions varied from

* Received for publication, January 10, 1947.

TABLE 1
SPLENIC ARTERIAL ANEURYSMS DURING PREGNANCY

CASE	AUTHOR	AGE	GESTATION	PATHOLOGIC FINDINGS	STATED PATHOGENESIS	OUTCOME
1.	Gillam	26	At term			Splenectomy, recovery
2.	Guy	25	5 months	Mitral valvulitis. Aneurysm 2 cm. in diameter at splenic hilum. Fibrosed wall. Normal artery.	Embolism	Rupture, death
3.	Danforth	28	7 months	Aneurysm of main artery, 1.5 cm. in diameter. Necrosis of wall.	Congenital	Rupture, death
4.	Lennie and Sheehan	30	8 months	Small false aneurysm of mid-artery. Thin wall. Recent infarcted spleen. Early atheromatous aorta.	Congenital	Rupture, death
5.	Lennie and Sheehan	30	8 months	Aneurysm of artery near spleen, 2 cm. in diameter. A wall of fibrous tissue and intima. Pancreatic hematoma.	Congenital	Rupture, death
6.	Sered and Steiner	30	Term	Small aneurysm of mid-artery. Mild sclerosis of artery.	Probable congenital	Rupture, death
7.	Bauer and Apfelmach	24	8 months	Aneurysm near hilum, 1 cm. in diameter. Atrophic media. Retrogressive changes.	Congenital	Rupture, death
8.	Yolland	27	4 months	Small aneurysm along main artery.		Rupture, death
9.	Smith ^a	35	9 months	Rupture of a branching of artery. No actual aneurysm.	Congenital	Rupture, death
10.	Landwall and Gödl	29	9 months	Aneurysm near celiac axis, 2 cm. in diameter.		Death after hysterectomy.
11.	Mayer	29	In labor	Aneurysm with a pancreatic hematoma, 3 cm. in diameter.		Rupture, death
12.	Remmelts	38	In labor	Aneurysm, 2 cm. in diameter.		Rupture, death
13.	Wesenberg	32	In labor	Aneurysm, 5 cm. in diameter.		Rupture, death
14.	Cosgrove, Watts and Kämp	29	5 months	Aneurysm near hilum, 4 cm. in diameter. Essentially normal artery.	Congenital	Rupture, death
15.	Cosgrove, Watts and Kämp	23	Postpartum	Two aneurysms near hilum, each 2.5 cm. in diameter. Necrotic wall.	Congenital	Rupture, death

Average age, 29 years

1 to 8 cm. in diameter and in many cases were multiple. Grossly, the splenic artery was tortuous and had intimal changes varying from slight thickening to large atheromatous plaques with ulceration and calcification. Atheromata and calcification were common in the aneurysmal wall and in long standing lesions little remained except thickened, friable and calcified material adherent to a laminated blood clot. These local changes were usually associated with general arteriosclerosis, especially of the aorta, the coronary and cerebral arteries.

TABLE 2
SITES INTO WHICH SPLENIC ARTERIAL ANEURYSMS RUPTURED IN 63 CASES

AREA	NUMBER
Lesser peritoneal sac and peritoneal cavity.....	32
Colon.....	5
Stomach.....	8
Retroperitoneal tissue.....	10
Body of pancreas.....	4
Stomach and peritoneal cavity.....	2
Stomach and transverse colon.....	2

TABLE 3
PATHOGENESIS OF SPLENIC ARTERIAL ANEURYSMS IN 63 CASES

PATHOGENESIS	CASES
Arteriosclerosis.....	37
Mycotic.....	12
Congenital.....	9
Miscellaneous	
Trauma.....	2
"Wandering" spleen.....	1
Gaucher's disease.....	1
Banti's disease.....	1
Total.....	63

Microscopically, intimal thickening, atheromata with patchy hyalinization and calcification of the media were common. General fibrosis of the wall was a constant feature.

In patients with mycotic aneurysms there was evidence of rheumatic valvulitis. The average age in this group was 24 years and there were as many males as females. Mycotic aneurysms varied from 2 to 3 cm. in diameter and were usually found in the finer branches of the splenic artery. Multiple splenic infarcts were found in 7 of the 12 cases, indicating their embolic nature. Microscopically, intimal thickening with hyaline degeneration of the media and fibrosis were constant findings.

In 9 of the 63 cases, some feature was present to indicate a congenital basis for the lesion. The average age was 35 years with females predominating. The

striking gross features were single saccular aneurysms varying from 1 to 7 cm. in diameter with the lesion most often found at or near branching points of the splenic artery. The splenic artery was usually normal or slightly thickened and often contained atheromata. In some instances, the sac displaced and was continuous with pancreatic tissue. Atrophic and defective medial tissue and internal elastic lamina with fibrosis and thinning of the wall were the prominent microscopic features.

TABLE 4
SYMPTOMS OF A CHRONIC NATURE ACCOMPANYING SPLENIC ARTERIAL ANEURYSMS
IN 25 CASES

-
1. A long history of upper abdominal pain (in left upper quadrant and/or epigastrium) often relieved, but in some cases aggravated by stooping, bending, or running
 2. Intermittent attacks of vomiting
 3. "Indigestion" simulating cholecystitis or peptic ulcer
 4. Loss of weight
 5. Anemia
 6. Diarrhea or constipation
 7. Occasionally dyspnea or syncope
-

TABLE 5
SYMPTOMS OF ACUTE NATURE ACCOMPANYING SPLENIC ARTERIAL ANEURYSMS
IN 40 CASES

-
1. Sudden onset of severe pain in upper part of abdomen, not easily relieved
 2. Melena and/or hematemesis, depending on whether the gastro-intestinal tract was penetrated
 3. Marked apprehension, often with dyspnea and syncope
 4. Repeated vomiting
 5. Pain in shoulder
 6. Urge for defecation
 7. Ultimate prostration, low blood pressure, rapid pulse and shock from intra-abdominal hemorrhage
-

DIAGNOSIS

In 65 of the 110 cases studied, the symptoms were readily arranged into those of a chronic nature (Table 4) and those of an acute nature (Table 5). Chronic symptoms varied in duration from one week to well over a year. In patients with symptoms of an acute nature a sudden leakage of blood from, or rupture of, the aneurysm was found. Many splenic arterial aneurysms ruptured in two stages.³² At first, there was a small rupture of the sac with a leakage of blood into the lesser peritoneal cavity, then a latent period, and finally a large rupture with massive and severe bleeding. The latent period varied from a few hours to eight weeks. In patients with premonitory symptoms surgical treatment may be instituted.

In addition to the symptoms already mentioned, the following diagnostic signs were found in the indicated number of cases: enlarged spleen, 22; palpable tumor, 13 (with pulsations, 5; with bruit, 5); pancreatic insufficiency (evidence

on examination of stool), 2; and x-ray evidence of aneurysm, 12. The roentgenographic shadow is usually sharply defined, being dense at the periphery and often mottled in the center. It is most commonly noted on a level with, and just to the left of, the first lumbar vertebra.³⁷

TABLE 6
CLINICALLY DIAGNOSED SPLENIC ARTERIAL ANEURYSMS IN 10 CASES

AUTHOR	BASIS FOR DIAGNOSIS	OUTCOME
1. Högler	Pulsating mass, bruit, fluoroscopic examination	Necropsy
2. Ortner	Enlarged spleen, bruit	Necropsy
3. Lindboe	X-ray	Successful surgical removal
4. Haffner	X-ray	Successful surgical removal
5. Säfwenberg	X-ray	Necropsy
6. Säfwenberg	X-ray	Necropsy
7. Guy	Pulsation, tumor and hemorrhage	Surgical removal
8. Sperling	X-ray	Surgical removal
9. Seids and Hauser	Mass, pulsation and x-ray	Surgical removal
10. Lower and Farrell	X-ray	Surgical removal

TABLE 7
SURGICALLY TREATED CASES OF SPLENIC ARTERIAL ANEURYSM IN 32 PATIENTS

	CASES	RECOVERED	DIED
A. Procedures directed to the aneurysm			
1. Splenectomy with removal of aneurysm....	13	11	2
2. Ligature			
proximal.....	2	2	0
distal.....	1	1	0
3. Tamponade.....	2	0	2
4. Ligation and removal of aneurysm.....	4	3	1
B. Procedures not directed to the aneurysm			
1. Exploratory only.....	6	0	6
2. Hysterectomy.....	1	0	1
3. Hysterotomy.....	1	0	1
4. Application of clamps.....	1	0	1
5. Laparotomy and cesarian section.....	1	0	1
Total	32	17	15

Sherlock and Learmonth found that the clinical diagnosis had been made in only 10 of the 124 cases they reviewed. In Table 6 are listed the 10 cases, the basis on which the diagnosis was made, and the outcome.

TREATMENT

The treatment of these lesions is essentially a surgical problem. In Table 7 are listed the surgical procedures attempted and the end results in the 32 cases so

treated. From this table it is obvious that the procedures of choice are those which are directed toward treatment of the ruptured aneurysm, preferably by ligation or resection.

Following is a summary of each of our three cases.

Case 1

Clinical data. A 29 year old white female was in her sixth month of pregnancy and had been in good health until a few hours before admission to the hospital, when she suddenly fainted. She was easily aroused but continued to be weak and pale. During the next hour, she fainted twice, began to have generalized abdominal pain, became much weaker and had a copious bowel movement without blood. She was acutely ill with cold, clammy skin and slight cyanosis of the mucous membranes and nail beds. She was well oriented. The temperature was 97 F., the pulse was weak and regular and the rate 140 per minute. The blood pressure obtained during the course of examination was once 68/0 and, several times, was unobtainable. Her abdomen was diffusely tender and extremely painful in the upper quadrants. Slight involuntary rigidity was present. There was dullness to percussion in the left flank which did not shift with change in position. No fetal heart tones were heard. The blood hemoglobin was 80 per cent, the leukocyte count was 16,000 per cu. mm. with 85 per cent neutrophils and 15 per cent lymphocytes. She was given oxygen by mask, 500 cc. of plasma and later glucose intravenously. The pain extended into her chest and up to the shoulders. Her blood pressure gradually became lower and she expired six and one-half hours after admission.

Gross findings. At necropsy, the peritoneal cavity contained 2000 cc. and the lesser peritoneal cavity 850 cc. of fresh and partly clotted blood. The kidneys were pale with numerous dense cortical scars and some edema. There was severe (grade 4 on the basis of grades 1 to 4) hydro-ureter on the right side and moderate (grade 2) hydro-ureter on the left side. The aorta was normal grossly, but small, and had a circumference of 4 cm. in the mid-abdominal portion. The spleen was soft and mushy in consistency and weighed 200 gm. The splenic artery was surrounded by clotted blood. At a point 10 cm. from the hilum, there was a sacular aneurysm 4 cm. in length with a mouth 0.5 cm. wide which opened into the artery (Fig. 1). The greatest diameter of the aneurysm was 2.5 cm. There was a tear 1.5 cm. long on the longitudinal axis of the aneurysmal sac surrounded by large blood clots, most of which appeared relatively fresh. In the remainder of the splenic artery there was no evidence of arteriosclerosis.

Microscopically, the structure of the splenic artery was normal (Fig. 3). As the border of the sac was approached, the muscular media became thinner; there was a considerable increase in the connective tissue component, and with special stains, evidence was found of fragmentation of the internal and external elastic laminae (Fig. 4). The wall of the sac was composed almost entirely of dense collagenous connective tissue with interspersed small fragments of elastic tissue (Fig. 5).

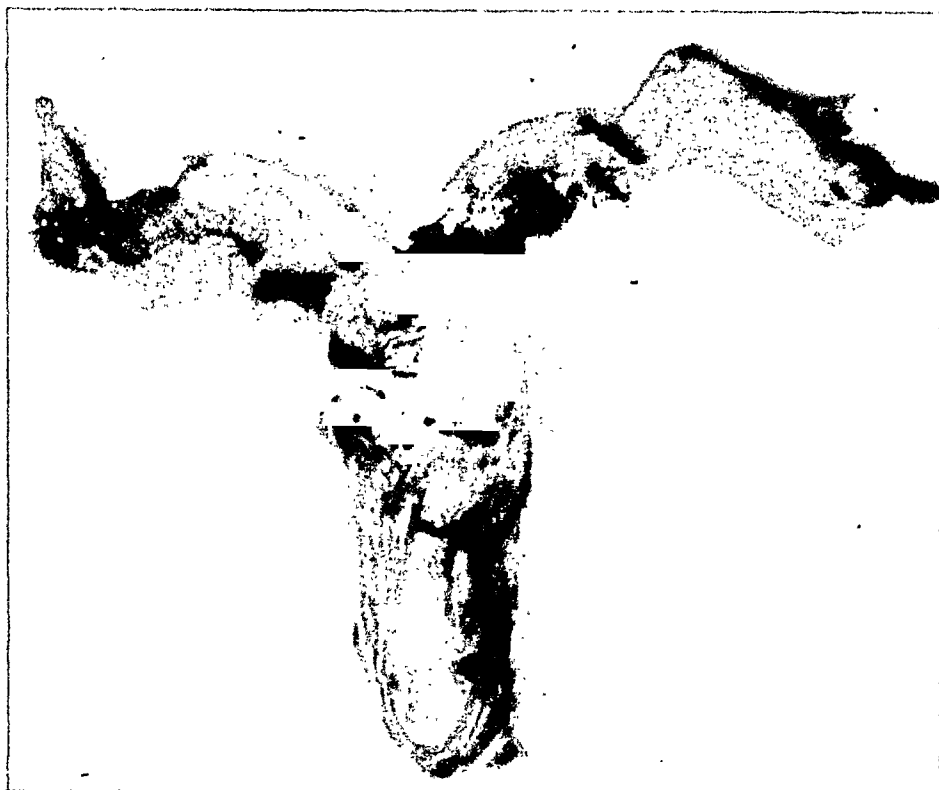
The final diagnoses were ruptured congenital aneurysm of the splenic artery, early splenic infarcts, pregnancy (five and one-half months), normal stillborn male, bilateral hydro-ureter, bilateral chronic pyelonephritis and atelectasis of lower lobes of both lungs.

Case 2*

Clinical data. A 23 year old white female was hospitalized in the evening, during the first stage of labor, with a history of frequent spells of syncope. At 2:15 A.M., the following morning, she spontaneously delivered a living female infant. The placenta was expressed without difficulty. There had been an increased blood loss with the delivery and she was given ergotrate intravenously and intramuscularly. Her general condition remained good

* The report of this case is presented through the courtesy of Dr. M. E. Maun, Wyandotte General Hospital, Wyandotte, Michigan.

until 6:15 A.M., when she suddenly complained of weakness, her skin became cold and she began to perspire profusely without losing consciousness. Her pulse was difficult to obtain and the blood pressure was 60/30 mm. of mercury. With stimulants there was some improvement and, at 10:00 A.M., the blood pressure was 90/60 mm. mercury. The uterus remained firm and uterine blood loss was normal. A small amount of urine was obtained by catheter. The blood hemoglobin was 84 per cent; the erythrocyte count, 4,410,000 per cu. mm.; the leukocyte count 22,700 per cu. mm. with 88 per cent neutrophils. The urine had a trace of albumin and numerous erythrocytes. Soon her pulse again became weak and irregular and she noted epigastric pain which radiated to the left side. At 4:00 P.M., she had a minor convulsion for which plasma and magnesium sulfate were given



EXTERNAL SURFACE OF THE SACCULAR ANEURYSM FROM CASE 1. THE SAC IS STUFFED WITH COTTON AND THE TEAR IS CLEARLY SHOWN.

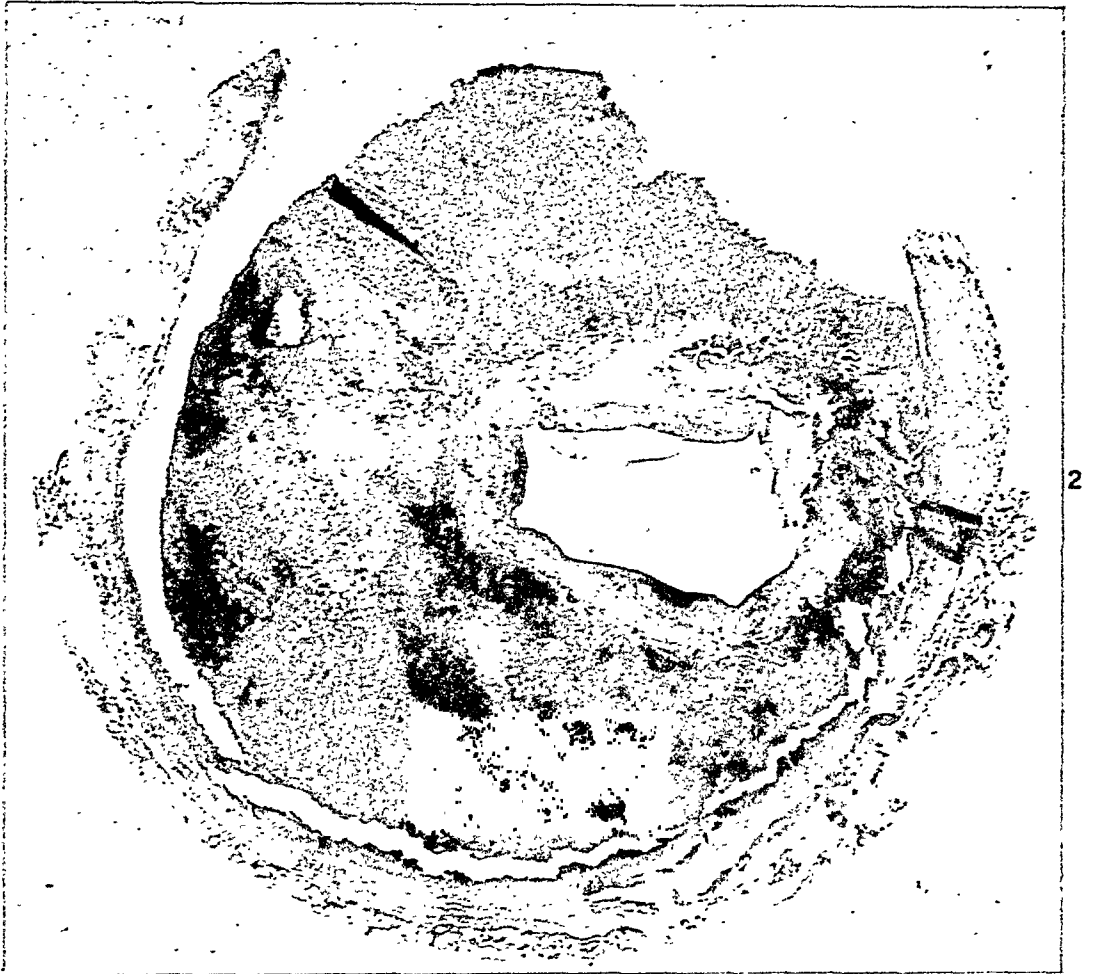
At this time there was tenderness to palpation in the epigastrium. At 5:15 P.M., she had a major convulsion, became pulseless, cyanotic and expired.

Gross findings. At necropsy, there was a left hydrothorax of 100 cc. In the peritoneal cavity there was 2000 cc. of blood with numerous large clots. The omentum and root of the mesentery were hemorrhagic and a massive hematoma 25 cm. in diameter was found in the lesser peritoneal sac near the spleen and partly covering the stomach. The uterus was of normal postpartum character. The spleen weighed 950 gm. and had a smooth tense capsule. It was bluish gray in color. On cut section, it presented a purplish red appearance and was soft in consistency. On the splenic artery there were two aneurysmal dilations about 5 cm. from the hilum of the spleen. The aneurysms were similar in appearance, each was about 2.5 cm. in diameter, and both were ruptured. The walls of the aneurysms were thin, dark red, rough and friable. The splenic vein contained a small firm thrombus.

Microscopically this lesion was essentially similar to that in Case 1.

Case 3

Clinical data. A 62 year old white male laborer was admitted to the traumatic surgical service for a laceration of the chin and fractured jaw. The physical examination was normal except for his blood pressure which was 175/90 mm. of mercury. The blood hemoglobin was 14.2 gm.; the erythrocyte count, 4,900,000 per cu. mm.; the leukocyte count, 6,900 per cu. mm. with 89 per cent neutrophils and 11 per cent lymphocytes. The urinalysis revealed a specific gravity of 1.022, a trace of albumin and occasional leukocytes. The



PHOTOMICROGRAPH TO ILLUSTRATE THE DISSECTING CHARACTER OF THE SPLENIC ARTERIAL ANEURYSM IN CASE 3. $\times 14$.

jaw was wired and the loose teeth removed. While under anesthetic his respirations became weak and irregular and he was given 3 cc. of metrazol. He reacted slowly from the anesthetic. His blood pressure and pulse were satisfactory but his urinary output was small. He was given oxygen, metrazol and intravenous glucose and saline. His blood nonprotein nitrogen was 62 mg. per 100 cc. Two days later he was improved and his urinary output was satisfactory. On the sixth hospital day he complained of a rather severe left intercostal pain. His blood pressure was 210/90 mm. mercury, pulse rate 104 and respiratory rate 24 per minute. He soon became lethargic and difficult to arouse and had marked physical weakness. He became progressively weaker, dyspneic, irrational and started to vomit. His blood nonprotein nitrogen rose to 144 mg. His cerebrospinal fluid was normal. He became comatose and expired on the sixteenth hospital day.

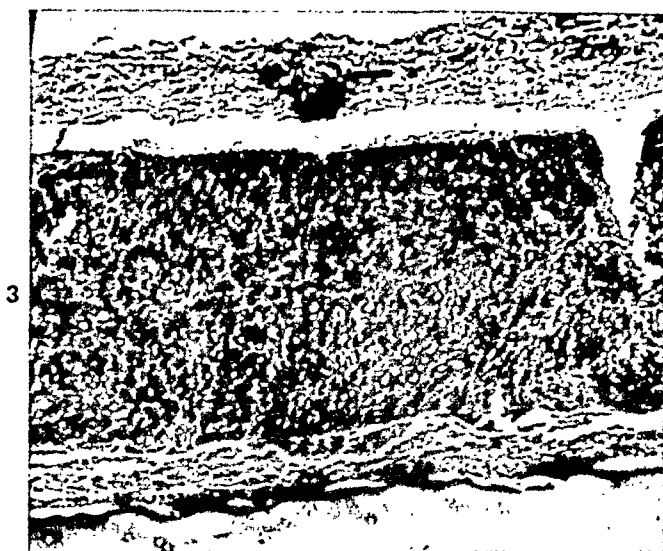


FIG. 3. Case 1. Photomicrograph of the Splenic Artery. $\times 125$.

FIG. 4. Case 1. Photomicrograph of the Border of the Aneurysmal Sac with Faulty Muscular Wall. $\times 125$.

FIG. 5. Case 1. Photomicrograph of the Sac Wall Composed Almost Entirely of Collagenous Connective Tissue with Small Remaining Fragments of Muscle and Elastic Fibers. $\times 125$.

Gross findings. At necropsy, there was 50 cc. of fluid in the left pleural cavity. Both lungs were congested and there were enlarged hilar lymph nodes on the right side. In the peritoneal cavity, there was a large amount of thin bloody fluid bathing the abdominal organs. The omentum and mesentery were hemorrhagic. There was a large blood clot which extended from the brim of the pelvis to the foramen of Winslow where a larger clot was found. The spleen weighed 175 gm., was soft in consistency and its cut surface was brownish, mottled and fibrous. There were mottled infarcts up to 2 cm. in diameter. In the midportion of the splenic artery there was a dissecting aneurysm with thrombosis and occlusion (Fig. 2), with distention of the outer diameter to 2.5 cm. At the proximal end of the obstructed area there was a small rupture of the wall of the vessel into the lesser peritoneal cavity. In the thoracic and abdominal aorta there was a moderately severe atherosclerosis (graded 3, on the basis of grades 1 to 4).

Microscopically, there was degeneration in the medial portion of the splenic artery. In one area, the media was completely separated by hemorrhagic extravasation. There were also many areas of intense degeneration and loss of staining qualities.

SUMMARY

We have reviewed 107 cases of splenic arterial aneurysm and have added 3 of our own.

We have indicated various pathogenetic factors in these lesions and have presented the gross and microscopic findings. Our survey indicates a frequent coincidence of ruptured splenic arterial aneurysm and pregnancy but no pathogenetic relationship. This review indicates that it is possible to make the diagnosis of ruptured splenic arterial aneurysm in life and that surgical intervention is frequently successful.

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IDIOPATHIC FIBROLYTIC DEGENERATION OF THE MENINGEAL DURA*

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The term idiopathic fibrolytic degeneration of the meningeal dura was chosen as the name of a condition considered to be a primary pathologic process characterized by a layer-like area of degeneration located in the inner or meningeal half of the dura mater. This lesion would be of little consequence if it were not for its complications of intradural hemorrhages and intradural hemorrhagic cyst formation which is chronic and progressive. It resembles idiopathic medial necrosis of the aorta which is generally recognized as a primary pathologic process and has a similar layering of degenerative products with disappearance of cellular elements, cyst formations and dissecting hemorrhages.

The literature on the general subject of dura hematoma has been reviewed elsewhere.⁹ The pachymeninx is made up of two embryologic layers which are said to fuse in the second month of intra-uterine life. The layer adjacent to the skull is termed the endosteal and that next to the arachnoid membrane, the meningeal layer. Large arteries such as the middle meningeal artery and its many branches are incorporated in the fibers of the endosteal dura. In the meningeal dura one can frequently find small arteries and giant capillaries. At times, a delicate vascular network can be found on the arachnoid surface of the meningeal dura. The fibers of the two layers of the dura generally run in different directions so that they can usually be easily distinguished histologically. Occasionally in adults, and more often in infants, one can find a dura whose two layers can be separated for short distances without much difficulty. Much more often, however, the layers are closely fused together. With advancing age, there is a gradual loss of cellularity and vascularity associated with an increasing hyalinization of the collagenous, fibrous connective tissue forming both layers of the dura. This is a natural aging process seen in varying degrees in connective tissue.

REPORT OF CASE

Clinical data. A 65 year old storekeeper sustained a fractured nose and an ecchymosis about the right eye two and one-half years before his death. Following the injury, he developed a decided character change which was characterized by a considerable accentuation of the alcoholic habit, loss of ambition and development of a disagreeable personality and a rapid succession of financial failures. During this two and one-half year period, he was admitted on three occasions to hospitals because of attacks of severe vomiting which lasted from two to six days. These attacks were considered to be due to intestinal obstruction, but he refused laparotomy. He was suffering from a similar attack at the time of his last admission to the hospital.

* Presented at the Twenty-Fourth Annual Meeting of the American Society of Clinical Pathologists, San Francisco, California, June 29, 1946. Received for publication, September 28, 1946.

Gross findings. At necropsy, a huge, chronic, intradural, hemorrhagic cyst over the right cerebral hemisphere (Fig. 3) and a large flat simple cyst in the left frontal area were found. The large hemorrhagic cyst extended from the most anterior portion of the frontal region to the most posterior portion of the occipital region and involved about one-half of the distance between the sagittal sinus and the base of the skull along this distance. The cyst varied from 1 to 2.5 cm. in thickness and its walls averaged about 1 mm. in thickness. It was filled with a blood stained, xanthochromic, rather thick, watery fluid. It also contained about 20 cc. of small, dark red, blood clots all of which appeared to be of recent origin. This recent hemorrhage was the apparent immediate cause of death. The surface layer adjacent to the arachnoid was continuous with the surface layer of the meningeal dura, while that portion of the cyst wall adjacent to the endosteal dura was continuous with the other half of the meningeal dura. There was a complete separation of the meningeal and endosteal portions of dura underlying the entire cyst so that one could easily break through the meningeal dura at any point along the margin of the hemorrhagic cyst and expose the normal appearing underlying endosteal dura.

A flat cyst 6 cm. in diameter and 5 mm. in depth was located in the meningeal dura in the left frontal area. Its clear watery fluid could be squeezed readily into adjacent parts of the dura in which the layers had appeared grossly intact. There was a mottling of light brownish yellow and red throughout the dura on both sides, due to the presence of old blood pigments, shown histologically to be in phagocytes in the meningeal layer.

Incidental necropsy findings in this case included a grade 2 cirrhosis of the liver and mild degenerative changes in the myocardium, liver, spleen and kidneys. There were adhesions between the duodenum and bed of the gallbladder and some dilatation of the lumen of the duodenum, but no evidence whatever of obstruction of any part of the small or large intestines. There were no diverticula, tumors, or areas of inflammation in any part of the gastrointestinal tract.

Microscopic findings. Many sections from scattered areas of the pachymeninx revealed a layer-like degenerative process generally located between the outer two-thirds and the inner one-third (adjacent to arachnoid) of the meningeal dura (Fig. 2). This degeneration was obviously slow and was characterized by a gradual disappearance of the fibroglia and later of the fibroblasts in the involved area, resulting in an acellular or poorly cellular, hyalinized space. Into this area occurred numerous small hemorrhages of different ages. Many pigment-laden phagocytes containing iron and small clumps of intact red blood cells of recent hemorrhages were present. These changes were found in many sections from the dura. A later stage in the process resulted in a splitting of the layers with cyst formation and/or large hemorrhages with splitting of layers (Fig. 4) and hemorrhagic cyst formation. There was practically no inflammatory cell reaction in any of the sections. All of the sections taken from the margin of the cyst revealed the same histologic changes which were limited to the meningeal dura. The two sides of the cyst were greatly thickened, hyalinized and poorly cellular and gave an index of the age of the process. At the inner margins were giant capillaries and iron pigment-laden phagocytes with beginning organization of the blood clot in the cyst. These naked capillaries were the obvious source of repeated hemorrhages into the cyst cavity. There was surprisingly little inflammatory cell reaction.

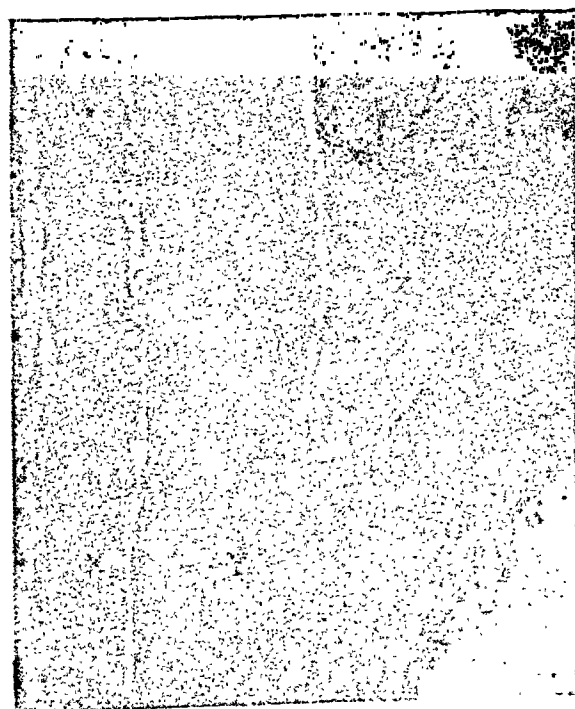
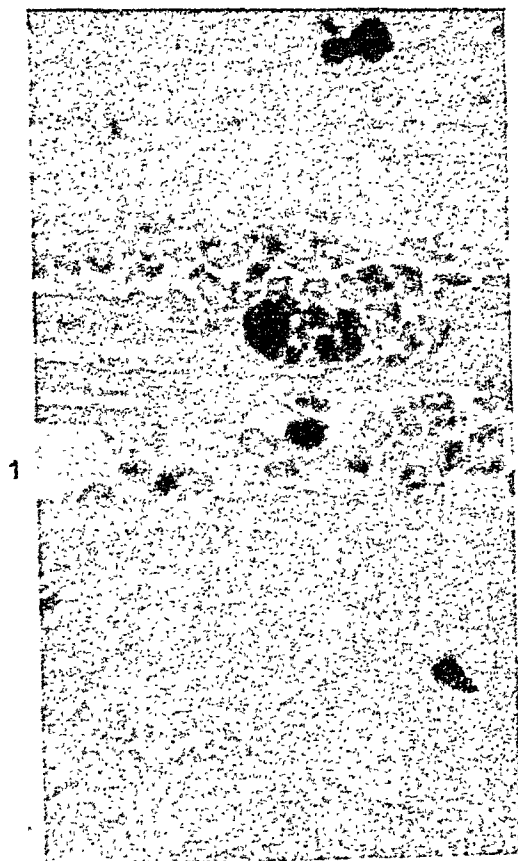
In addition to the large cyst found in the dura from the left frontal area, other very small cystic spaces were found in microscopic sections taken from various parts of the dura from both sides of the cranium at sites of fibrolytic degeneration.

FIG. 1. High power view of degenerated area of meningeal dura with recent hemorrhage and pigment-laden phagocytes.

FIG. 2. Layer-like degeneration of the meningeal dura, pigment-laden phagocytes, giant capillary and endosteal dura.

FIG. 3. Portion of intradural hemorrhagic cyst with separated margins exposing endosteal dura, and recent blood clots in opened cyst cavity.

FIG. 4. Margin of hemorrhagic cyst. X is normal endosteal dura, Y is thickened meningeal dura and Z is organizing blood clot at inner margin of cyst.



DISCUSSION

In 1914, Korwitz⁵ and later Hannah,² Kaump and Love⁴ and Baker¹ described hemorrhagic cysts of the dura as true intradural hematomas. In fact, Baker was of the opinion that there is never a fibrous organization with cyst formation resulting from subdural hemorrhages and that the blood, both free and clotted, from such hemorrhages, is eventually absorbed. To my knowledge, a typical chronic hemorrhagic cyst has never been produced experimentally and is not found after craniotomy. The great majority of neurosurgeons and pathologists writing on the subject of "subdural hematomas" describe the condition as the result of subdural hemorrhage with fibroblastic organization and a subsequent cyst formation. Timothy Leary⁶ has verbally proved that intradural hematomas are impossible. His colleague, Munro,⁷ who has reported 310 cases of "subdural hematoma," feels that no one should write on the subjects of clinical manifestation, treatment, or pathology of these hematomas unless he has 100 or more cases to report and that fewer cases would not add to the present accepted knowledge of these subjects. I do not have sufficient experience with chronic hemorrhagic cysts of the dura to say whether they develop from subdural hemorrhage. That self-perpetuating, chronic hemorrhagic cysts do occur as a result of intradural hemorrhages is beyond question. During the last year, I have seen six instances of intradural hemorrhage. Only one of these was associated with the fibrolytic degeneration of the dura. Four of the others had unusually advanced hyalinized changes in the meningeal dura associated with what appeared to be a tendency for the layers to split parallel to the surface. Only two of these dural hemorrhages were large enough to cause death, and these two were associated with multiple small hemorrhages at scattered points in the dura. In only one of the 6 cases had the hemorrhages formed a true chronic hemorrhagic cyst. It is obvious that there are other causes of intradural hemorrhages besides fibrolytic degeneration.

Simple cysts located in the dura mater are indeed rare, since apparently only two cases have been recorded. One described by Voss,⁸ occurred in the dura of the spinal cord and its histogenesis was not apparent. The other, described by Haymaker and Foster,³ appeared to be a congenital failure of fusion of the embryonic layers of the dura and occurred in the occipital fossa. There are several cysts described as spatially related to the dura and principally resulting from congenital defects.

Inasmuch as an apparent error is being propagated in the literature concerning the histogenesis of chronic hemorrhagic cysts of the dura, the author wonders if there is not a similar error related to the rarely described "subdural hydroma." Theoretically, this is a collection of cerebrospinal fluid in the subdural space as a result of a break in the arachnoid membrane. Is it possible that a more careful examination might reveal this fluid to be enclosed in the thin split layers of the meningeal dura?

SUMMARY

A new pathologic entity, idiopathic fibrolytic degeneration of the meningeal dura, is described with its two complications, intradural hemorrhagic cyst and

intradural simple cyst: There are other causes of intradural hemorrhages and self-perpetuating hemorrhagic cysts. The need of a better understanding of the pathogenesis of dural hemorrhages is stressed in spite of the almost general agreement expressed in the present day literature on the subject.

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RELAPSING FEVER AT LAKE TAHOE, CALIFORNIA-NEVADA*

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Lake Tahoe, situated partly in California and partly in Nevada, about 50 miles southwest of Reno, Nevada, is probably the most important endemic focus of relapsing fever in the United States. An estimated³ 250,000 vacationists visit Lake Tahoe each year, coming in large numbers from June to September. They return to their homes at distant points in the United States and become perplexing diagnostic problems, since relapsing fever, although well known to the medical profession in California, Nevada and the other western states where it occurs, is comparatively unknown in the East.

Since 1935, the writer has examined blood specimens containing *Borrelia recurrentis* in from 6 to 12 different patients each season. This is undoubtedly only a small part of the annual number of cases, since many are diagnosed and treated in California, while others are either treated empirically, or are undiagnosed.

The relapsing fever season begins late in the spring and is usually finished by the late fall, although a few infections have been known to develop even during the winter months. Relapsing fever is known to exist in thirteen western states (Arizona, California, Colorado, Idaho, Kansas, Montana, Nevada, New Mexico, Oklahoma, Oregon, Texas, Utah and Washington), In British Columbia, and one case⁵ has been reported in Maryland. Most of the reported cases have been from California and Texas, with Big Bear Lake, in the San Bernardino mountains about 60 miles east of Los Angeles, and Lake Tahoe as the most important endemic foci in California. Briggs,² reported the first cases of relapsing fever in California, in the summer of 1921. The disease was acquired on the Truckee River, about 20 miles from Lake Tahoe. Magee,⁶ then a student of Briggs at the University of California Medical School, encountered the infection in patients from Lake Tahoe, in the summer of 1934. I am indebted to him for learning of the existence of the disease there.

The excellent monograph¹ of the American Association for the Advancement of Science can be highly recommended to those interested in all phases of the subject. The spirochetes, *Borrelia recurrentis*, causing the disease, are harbored in chipmunks and gray tree (tamarack) squirrels abounding in the environs of the Lake. The disease is transmitted to man by the bite of certain *Ornithodoros* ticks living upon these rodents, chiefly *O. hermsi* and *O. parkeri*. *O. hermsi* is small, measuring about 6 by 3 mm., and its bite is seldom noticed by man owing to its characteristic feeding habits. The tick often feeds nocturnally, does not bury its mouth parts into the skin as do ordinary wood ticks (genus *Dermacentor*), feeds from a few minutes to an hour, drops off after feeding and, ordinarily, leaves little or no local reaction at the site of the bite. Occasionally, a well marked area

* Received for publication, February 8, 1947.

of erythema and a wheal appear, but usually the patient with the disease cannot recall having been bitten by any insect.

The onset of the disease is sudden, following an incubation period of about one week after the tick bite. Chills, fever ranging from 100 F. to 106 F., with an average maximum of 104 F., generalized aching, malaise, severe headache and sweating are the commonest symptoms. Nausea and vomiting occur frequently. These symptoms are often mistaken for an attack of influenza, or upper respiratory infection, but a knowledge of the seasonal occurrence of the disease and its geographic distribution will lead one to suspect relapsing fever and search for the spirochetes in the blood. The organisms are present during the febrile period and rarely are found during the afebrile interval. The fever lasts from two to five days and usually stops abruptly. There is an afebrile period of approximately six to ten days during which the patient usually feels well. Then, in untreated patients and, occasionally, in treated patients, a sudden febrile relapse occurs. Sometimes, the patient suggests the diagnosis to his physician by saying, "I had the same symptoms at Lake Tahoe about a week ago." In untreated subjects, the disease lasts about three months, the febrile paroxysms gradually becoming milder and shorter until the disease finally burns itself out.

Physical examination usually reveals nothing distinctive. Slight enlargement of the spleen is present in about one-third of the patients. Jaundice is not common, but the writer recalls one man who was markedly jaundiced, very ill, and had a leukocyte count of 15,000 per cu. mm. These findings are unusual. In his blood smears, the spirochetes were quite numerous, one or two being generally found in a single field under oil immersion, which is also an uncommon finding in patients infected at Lake Tahoe. A macular rash on the extremities is rare in the California cases, but it occurs in about 50 per cent of infections in Texas. The pulse rate is usually moderately accelerated. Some patients have a dry, red throat which, with the râles occasionally heard, may lead to an erroneous diagnosis of upper respiratory infection or influenza. A little albumin and some casts in the urine are commonly found and represent no contraindication to arsenical therapy.

Special mention of the leukocyte count should be made. I have found that in the vast majority of cases in which blood counts were made, the number of leukocytes varied between 7,000 and 9,000 per cu. mm., with no increase in neutrophils and no shift to the left. I have not infrequently suspected relapsing fever in patients visiting at Lake Tahoe during the summer months, who had a high fever and a normal white blood cell count. In fact, Morrison⁷ was led to suspect the disease before the beginning of the usual season in May, because of the fever and the normal leukocyte count.

LABORATORY DIAGNOSIS

Borrelia recurrentis is an actively motile blood spirochete of variable length, about 10 to 30 microns, having fairly wide spirals which vary from three to twelve in number. It closely resembles *B. vincenti* in fresh and stained preparations. It is rarely found during the afebrile intervals and sometimes is difficult

to find in febrile patients, even when the fever is at its height. In striking contrast, large numbers of spirochetes are readily found in the disease as it occurs in India, China and Africa. The organism stains readily in blood smears by Wright's stain or other blood stains.

In the fresh state, it is highly motile. Motility is not only axial, but the organism frequently doubles upon itself and darts across the dark field so that it is often difficult to keep it in vision. In fresh blood specimens, the motility persists for about four to six days at room temperature. Thus, it is possible to keep a test tube of clotted blood on hand for purposes of demonstration. In rare instances, I have found that the organisms will disappear overnight in serum of clotted blood. For this reason, it is advisable to make dark field examinations of fresh blood specimens only and to prepare several smears of the blood at the same time. The writer has failed, on two occasions, to find the spirochetes by dark field examination of a clotted specimen of blood on the day following their demonstration in stained smears by a technician. The routine practice in this laboratory is to prepare several rather thick smears by the two-slide method, stain with Wright's stain and examine them while the blood is clotting in the bacteriologic incubator. The spirochetes are often found first in the stained smears, but many times the dark field yields a positive diagnosis when either no organisms, or very few organisms, are found in the smears after a long search.

After ten years of experience, the writer is convinced that dark field examination offers the quickest and simplest means of finding the organism. Thin blood smears stained with Wright's stain are the least satisfactory, as the organisms are either not found at all, or else are found only after a search of an hour or more. Thick drop preparations are time-consuming, are not always positive and are unnecessary when the dark field method is used. When the patient is not available for securing a small amount of fresh capillary or venous blood, however, rather thick blood smears frequently are satisfactory, but often a considerable search is required before the first spirochete is found.

For dark field work a small amount of fresh whole capillary or venous blood is allowed to clot in a test tube. Anticoagulants should not be used. Clotting may be hastened in the incubator at 37 C'. A small loopful of serum with a moderately heavy red cell suspension is transferred to the extra thin microslide and a #1 cover glass is pressed gently upon it in order to prevent floating of the cover glass and to avoid convection currents which may carry the spirochetes out of the field. A moderate number of erythrocytes in the oil immersion fields is desirable to aid in focusing. Large numbers of erythrocytes may obscure the finding of spirochetes, but their agitation by the highly motile organism often directs attention to its presence.

The waving, undulating, threadlike objects, apparently fibrin threads, often seen in dark field examination of blood, whether containing spirochetes or not, cannot be mistaken for *B. recurrentis*, which is so highly motile as to be unmistakable. Frequently, the organisms are found quickly, but one half hour's search may be necessary, on rare occasions, particularly in instances in which there have been several relapses. Clear blood serum, which has been removed

from the clot, may be centrifuged so as to concentrate the organisms. The supernatant portion is then poured off and the sediment mixed with a small loopful of blood for dark field examination. I have found this method helpful occasionally when organisms were not found without centrifuging.

Animal inoculation has not been done in the writer's laboratory. Undoubtedly the intraperitoneal inoculation of whole blood into mice is advantageous from a scientific standpoint, but its practical use is questionable. The patients are usually very sick and a quick diagnosis is demanded. The incubation period in mice may be as long as twelve days before the spirochetes are demonstrable in preparations from the tail blood. This is an obvious drawback to early diagnosis. In this locality, either the organisms are found by the dark field method or the patient is given a therapeutic test.

PROGNOSIS AND TREATMENT

To my knowledge, no fatalities have occurred among the patients infected at Lake Tahoe. The disease gradually diminishes in severity in untreated patients in about three months. As a rule, the infection is very susceptible to the various arsenic compounds used in the treatment of syphilis. Neoarsphenamine administered intravenously is the drug commonly employed. The dose usually given is 0.45 gm., although some physicians use 0.6 gm. To be effective, it must be given during the febrile period, since relapses are commoner when the drug is given during the period of remission. Occasionally, relapses occur even when treatment is instituted during the fever, but this is uncommon. In such instances, the higher dose, 0.6 gm. is advised, care being taken to administer it as early in the febrile period as possible. Mapharsen has also been quite successful and is given intravenously in a dose varying from 0.03 to 0.06 gm. Sulfarsphenamine is used for infants and for those patients whose veins are too small to inject. The dose for infants and children is 0.01 gm. per kg. of body weight and the usual single full dose for adults is from 0.4 to 0.6 gm. As a rule, the first dose of the above drugs stops the disease promptly and recurrences are uncommon. Except for rather rare ocular complications, the prognosis is excellent.

Heilman and Herrell⁵ have proved the effectiveness of penicillin in the eradication of *B. recurrentis* from the blood of experimentally infected mice after four days of continuous treatment. Fischer⁴ has reported a case of a patient who had had four relapses and was cured after four days of treatment with penicillin. His patient was given intramuscular injections of 60,000 units at three hour intervals and remained well seventy-two days later. The writer suggests four or five days of similar penicillin therapy in the rather rare instances in which relapses occur in spite of several intravenous injections of one of the arsenicals mentioned above.

SUMMARY

Lake Tahoe, in the Sierra Nevada mountains of California-Nevada, is probably the most important endemic focus of relapsing fever in the United States. It is

estimated that about 250,000 persons visit there annually in normal times. The disease is transmitted to man by the bite of small *Ornithodoros* ticks which live on chipmunks and gray tree squirrels. The causative organism, *Borrelia recurrentis*, is frequently difficult to demonstrate in blood smears from patients because of a paucity of organisms, but during the febrile period is easily found in dark field examination of blood serum. Neoarsphenamine and mapharsen administered intravenously are recommended for treatment, one dose usually effecting a cure. Penicillin is recommended for those patients in whom relapses occur in spite of arsenical treatment. No fatalities have been reported to date from this area.

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EDITORIAL

THE DEVELOPMENT OF CLINICAL PATHOLOGY AS A SPECIALTY OF MEDICINE

The organization of the American Society of Clinical Pathologists was the first step in the establishment of clinical pathology as a specialty of medicine. Since that time, we have seen clinical pathology gradually emerge through the years as a distinct and separate, but highly important, specialty. The morphologic pathologist has always been recognized as the final authority in anatomic diagnosis, but with the development of our knowledge of other fields, such as bacteriology, serology and biochemistry, we have been enabled to recognize abnormal function before changes in form occur. Thus, the greater adaptability of clinical pathology has led to a demand for more clinical pathologists and more clinical laboratories. The clinical pathologist is in a unique position because of his association with all branches of medicine. By virtue of this unique position, the pathologist, having acquired a well rounded education and experience, should come to occupy a place of leadership among his fellow practitioners.

In order to elevate the position of the pathologist, as well as the practice of clinical pathology, it is essential that the pathologist of the future be properly trained, that provisions be made for continued instruction after graduation, that pathologists' groups be thoroughly organized, that laboratories be certified and methods be standardized. In addition, a program of lay education should be developed to make the patient aware of the value of laboratory methods in the diagnosis and control of disease and make him more receptive to the need for laboratory studies.

FRANK W. KONZELMANN

DEVELOPING THE INVESTIGATIVE HABIT

Aside from the multiplicity of duties clinical pathologists are called on to perform, many of them have certain self-imposed obligations. We refer to the obligation for regular study, investigation and writing. By nature and temperament, most pathologists are inclined to pursue such endeavors as a part of their work. Those of us who fail to do so often excuse ourselves by saying we are "too busy," but, actually, we have merely not developed the habit. Obviously, if the desire to study, investigate and publish be sufficiently great, this habit, "the investigative habit," will be developed.

It would seem that every pathologist could arbitrarily set aside a given portion of each day or week, preferably during regular hours, for such endeavors. This effort should not be regarded as an indulgence in luxury, but rather as an essential scientific obligation which is voluntarily assumed. Such an effort may be initiated at any time, as part of one's regular schedule and, if persisted in, should quickly lead to stimulating and productive results.

Almost every pathologist could find sufficient time to study his material and

the related literature and, each year, write two or three case reports worthy of publication. As the "investigative habit" is developed, the pathologist should soon become engaged in some organized study and should also produce, each year, perhaps one serious paper suitable for publication.

The field of clinical pathology is in the vanguard of progress in medicine. Many advances in medicine come in by way of the laboratory where diagnoses are verified and methods of treatment are subjected to control. Consequently, the clinical pathologist holds a key position in the hospital and in the community. It is, therefore, particularly important for him to develop the investigative habit and report his own original observations. If he does not, he may well ask himself if he is measuring up to the responsibility which he assumes as a pathologist.

BOOK REVIEWS

Entstehung und Früherfassung des Portiokarzinoms. By DR. HANSJAKOB WESPI, Chefarzt der geburtshilflich-gynäkologischen Abteilung am Krankenhaus Frauenfeld. 183 pp., 94 figs. 18 francs. Basel: Benno Schwabe & Co., 1946.

This book deals principally with the diagnosis of early carcinoma of the portio vaginalis uteri as made by histologic studies and by examination through the colposcope. The author recognizes a number of histologic changes which finally may culminate in the classical carcinoma. Among the intermediary stages he differentiates: "simple atypical epithelium" (increase in prickle cells, superficial parakeratosis, hyperkeratosis), "disturbed epithelium" (few atypical cells, but slight increase of mitoses), "non-carcinomatous increasingly atypical epithelium" (atypical cells not characteristic enough to be regarded as carcinomatous), "surface cancer" (pre-invasive carcinoma or carcinoma *in situ*). The simple atypical epithelium, however, is not necessarily to be regarded as precancerous since it may be present in a number of conditions unrelated to cancer. Because of the fact that in many instances the various changes of the cervical epithelium have been recognized by the use of the colposcope and verified on histologic examination, the routine colposcopic examination of women over 30 is emphatically stressed.

A small portion of the book is devoted to considerations of the possible origin of carcinoma. From his studies of very early carcinomas, the author stresses the multicentric origin of the so-called "non-carcinomatous, increasingly atypical epithelium" and of early cancers. He takes this multicentric origin as evidence that healthy cells somehow are changed by adjacent tumor cells and thus develop into cancer cells. It is thought that perhaps a virus is responsible for such changes.

Many case histories are quoted and the histologic and colposcopic findings are given in detail. There are excellent photomicrographs to illustrate the author's classification of cellular changes. There are 176 references, but as would be expected, the more recent American literature, which was probably not available to the author, has been omitted.

Chicago

OTTO SAPHIR

Quantitative Clinical Chemistry. Interpretations. Volume 1, Ed. 2. By JOHN P. PETERS, M.D., M.A., and DONALD D. VAN SLYKE, Ph.D., Sc.D., Member of the Rockefeller Institute for Medical Research. 1041 pp., 62 figs., 40 tables. \$7.00. Baltimore: The Williams & Wilkins Company, 1946.

The first volume of the long awaited second edition of "Interpretations" lives up to its high expectations. This outstanding treatise, the first edition of which set a standard for books in clinical chemistry, has now been expanded into two volumes, of which only the first volume has been published. The present volume is divided into four parts: Part I, Energy Metabolism; Part II, Carbohydrates; Part III, Lipids; and Part IV, Protein Metabolism. The bibliography pertaining to these four parts contains more than 2300 references to the literature.

Owing to the rapid advances in clinical chemistry since the appearance of the first edition in 1932, the authors found it necessary to recompose the text *in toto*. The present volume is a valuable reference book for physicians interested especially in laboratory medicine and summarizes a great fund of information for the research worker in the related fields of medicine and biochemistry.

Philadelphia

F. WILLIAM SUNDERMAN

Mongolism and Cretinism. A Study of the Clinical Manifestations and the General Pathology of Pituitary and Thyroid Deficiency. By CLEMENS E. BENDA, M.D., Director, Wallace Research Laboratory for the Study of Mental Deficiency, Wrentham, Mass., Instructor in Neuropathology, Harvard Medical School, Assistant in Psychiatry, Massachusetts General Hospital, Lecturer, Postgraduate Seminar, Massachusetts Department of

Mental Health. 310 pp., 101 illus., 48 tables. \$6.50. New York: Grune and Stratton, 1946.

It is estimated that there are about 60,000 living mongoloids in the United States and that about 17 are born daily. A check of latest editions of six popular textbooks on pathology netted four lines on mongolism in only one of them. The other texts did not even mention it in the index.

Dr. Benda deserves commendation for a valuable contribution to a field of medical literature which has been sadly neglected.

The monograph is based on a study of 300 patients over a period of ten years and includes fifty autopsies. Cretinism has been included mainly as a means of comparing a much better known disease, due to thyroid deficiency, with a less well known disease due, according to Benda, to congenital hypopituitarism. A considerable number of new details regarding cretinism has been added. The subject is presented with great detail in twelve chapters, the headings of which reveal the extent of the material to be found: history, physical characteristics, mental development, nervous system, endocrine pathology (thyroid, pituitary, gonads and suprarenals), general pathology (especially valuable for pathologists), growth and development of skull and bones, x-ray studies, hematology and biochemistry (much information not easily found elsewhere), relation of mongolism to the maternal condition, prevention of mongolism, and principles of treatment. Mongolism and cretinism are discussed in each chapter.

It is not at all certain that the basic concept of mongolism being caused by hypopituitarism is correct. Further investigation may support or disprove this concept without affecting the basic value of the book as a complete summary of present day knowledge of the condition.

There are excellent summaries following each topic. The monograph reflects great erudition and much original investigation and thinking. The bibliography and indexes are complete and well organized and the external appearance is pleasing.

Chicago

I. DAVIDSOHN

Clinical Hematology. Ed. 2. By MAXWELL M. WINTROBE, M.D., Ph.D., Professor of Medicine, University of Utah, School of Medicine, Salt Lake City. 862 pp., 197 eng., 14 plates, 10 in color. \$11.00. Philadelphia: Lea and Febiger, 1946.

The author has brought up to date the first edition of his book which has already become established and accepted as the standard text and reference book in hematology. The material on the erythrocyte has been expanded to include a chapter in discussion of the production and destruction of the red corpuscles. Of particular interest is the section on folic acid with an evaluation of this drug up to the date of publication. The chapter on general considerations and treatment of anemia is enlarged to include comments on substances other than iron. The author succinctly describes the management of polycythemia, using radioactive phosphorus. A discussion on the clinical effects of nitrogen mustard in the treatment of the lymphomatous diseases is included with particular evaluation in patients with Hodgkin's disease. The chapter on anemia in infancy and childhood is enlarged with special reference to the recent publications and newer knowledge of the Rh factor and erythroblastosis fetalis. As another example of the author's efforts to bring his book up to date, he has commented on the use of globulin fraction I in the treatment of patients with hemophilia. Descriptions of such recently described diseases as acute infectious lymphocytosis are also included.

The bibliography at the end of each chapter refers to the most important contributions on the subjects presented. The reviewer regards this book as the most complete and comprehensive published in the field of hematology up to the present time.

Brooklyn, N. Y.

LEO M. MEYER

An Atlas of the Commoner Skin Diseases. Ed. 3. By HENRY C. G. SEMON, M.D., D.M. Oxon., F.R.C.P., Physician for Diseases of the Skin and Lecturer to Postgraduates,

Royal Northern Hospital, Consulting Dermatologist, Hampstead and North-West London General Hospital. 339 pp., 139 color plates. \$12.00. Baltimore: The Williams & Wilkins Company, 1946.

This atlas contains 139 Kodachromes of skin lesions taken from living subjects. The excellence of the plates and the "life interest" invite one to scan all of its pages at one sitting. While the volume has more particular appeal and practical value for the clinician than for the pathologist, the latter will also find many photographs to arrest his attention. For the practitioner, it certainly can be recommended.

The general pathologist would welcome an atlas on dermatologic histopathology possessing similar excellence in photography, if the photomicrographs were accompanied by accurate descriptions of the essential lesions.

S. E. G.

Concise Chemical and Technical Dictionary. By H. BENNETT, Technical Director, Glyco Products Company, Inc., Fellow American Institute of Chemists, American Association for the Advancement of Science, Editor-in-Chief, The Chemical Formulary, Practical Emulsions, Commercial Waxes. 1055 pp. \$10.00. Brooklyn, N. Y.: Chemical Publishing Company, Inc., 1947.

This dictionary of 1055 double-columned pages has a pleasing make-up. The terms to be defined are set in bold type and the definitions are concise and easily read, the second and succeeding lines each being indented. Chemical formulas and chemical properties are included wherever indicated. Also included are tables listing the Greek alphabet, Greek symbols used in chemistry, mathematical symbols, tables of weights and measures, equivalent measures, chemical elements and their discoverers, temperature conversion tables, indicators, vitamin values of foodstuffs, structural formulas of important organic ring systems (covering 19 pages), and an addendum of recent terms.

The pathologist will find in this handy volume a great deal of useful information not available in standard medical dictionaries.

NEWS AND NOTICES

INTERNATIONAL HEMATOLOGY AND Rh CONFERENCE

The International Hematology and Rh Conference was held in Dallas, Texas, on November 15 and 16, 1946, under the sponsorship of Baylor Hospital, and in affiliation with the Second Mexican Congress of Blood Transfusion. The opening sessions were held in Baylor Hospital, Dallas, Texas, and the closing sessions at the Juarez Hospital, Mexico City. The officers for the meeting were Dr. Joseph M. Hill, chairman, and Dr. Sol Haberman, secretary, for the Dallas sessions, and Dr. Eduardo Uribe Guerola, president, and Dr. Alfonso Velez, secretary, for the Mexican Transfusion Congress. The meetings were open to members of the medical profession and to workers in the biologic and chemical sciences interested in the study of blood. English and Spanish were the official languages at both meetings and the services of interpreters were offered to those having language difficulties. The United States was heavily represented by registrations from 21 States and the District of Columbia. There were 16 registrants from Mexico, 2 from Canada, 1 from England and 1 from China. Difficulties in obtaining transportation prevented invited guests from Greece, Italy, Poland, Argentina and Colombia from attending the meetings.

The program of the International Hematology and Rh Conference in Dallas was as follows:

A Brief Survey of the Rh Factor.—Philip Levine, Raritan, N. J.

Interrelationship between the Rh System and the A-B System.—Ernest Witebsky, Buffalo, New York

The Nucleolar Structure of the Blood Cells.—Ignacio Gonzales Guzman, Mexico City

The Rh Genotypes and Fisher's Theory.—Robert R. Race, London, England

Rh Hemolytic Immune Globulins: Evidence for a Possible Third Order of Antibodies Incapable of Agglutination or Blocking.—Joseph M. Hill and Sol Haberman, Dallas, Texas

Hemolytic Mechanisms.—William Dameshek, Boston, Mass.

Physiochemical and Immunological Character of the Rh Antibodies.—Louis K. Diamond, Boston, Mass.

Rh Antibodies.—Israel Dayidsohn, Chicago, Ill.

The Frequency of the Rh Factor in Different Groups of the Mexican Population.—Mario Salazar Mallen, Mexico City

Variation in the Outcome of Pregnancies in which Erythroblastosis Occurs.—Bruce Chown, Winnipeg, Manitoba, Canada

The conferees then flew, on November 17, to Mexico City to attend the Second Mexican Blood Transfusion Congress, which was held in the Palace of Fine Arts, with members from Canada, England and the United States as honorary guests of the Assembly of Surgeons. Addresses of welcome were made by Dr. Miguel Lavalle, President of the National Assembly of Surgeons, Dr. Salvado Zubiran, Rector of the National University of Mexico, and by Dr. Gustavo Baz, Secretary of the Department of Public Health and Assistance of Mexico.

During the meeting, visits were made to the Hospital of Jesus, which is the oldest hospital in the Western Hemisphere, having been in operation since 1554, and to the Military Hospital, which is the most modern hospital in Mexico. The members were officially received by the National Academy of Surgery on November 19, and on November 21, by Dr. Francisco Castille Najera, the Minister of Foreign Affairs.

The following program was presented in both Spanish and English:

Production and Use of Anti-Rh Serum.—J. M. Hill and Sol Haberman, Dallas, Tex.

The A and B Factors as Possible Causes of Erythroblastosis.—Alfonso Velez Orozco, Mexico, D. F.

Demonstrations of Technics of Rh Testing.—Sol Haberman and J. M. Hill, Dallas, Tex.
Subgroups of the Rh Factor. Demonstration of the Genetics of Rh and Other New
Blood Groups.—R. R. Race, London, England

Percentages of the Rh Subgroups in Mexico.—A. V. Orozco and Rolando Medina Aguilar,
Mexico, D. F.

The Individuality of Human and Animal Blood.—Philip Levine, Raritan, N. J.

The Importance of the Rh Factor and Historical Developments.—Philip Levine, Raritan,
N. J.

The Use of ACD Solution in Blood Banking.—R. Medina Aguilar, Mexico, D. F.

The Histopathology of Erythroblastosis.—I. Gonzales-Guzman, Mexico, D. F.

The Treatment of Erythroblastosis by Complete Exchange Transfusion (motion picture).
—Harry Wallerstein, New York, N. Y.

On November 23, the concluding sessions of the meetings were held with Dr. Eduardo Uribe Guerola, President of the Mexican Transfusion Congress, and Dr. Joseph M. Hill, Chairman of the International Hematology Conference, presiding as co-chairmen. The following recommendations were adopted:

1. The term fetal erythroblastosis is to be retained temporarily for cases resulting from isoimmunization of mothers by Rh, Hr and other blood factors.

2. Use of the terms Rh-positive and Rh-negative along with homozygous and heterozygous is to be retained for clinical use.

3. A committee for the study of terminology of blood antigens was appointed by the chairmen of both meetings. The committee was charged with the responsibility of making recommendations for standardization of terms for these antigens and their subgroups. Both organizations strongly recommended that the terms glutinins, X-protein and conglutination be withheld from acceptance until further study is made. The Fisher-Race theory of inheritance in nomenclature of the Rh-Hr subgroups was recommended for temporary acceptance until the problem can be reviewed. It was concluded that, in the light of our present knowledge, the Fisher-Race theory, using the CDE system of classification, was most suitable for serologic and genetic study of the Rh-Hr subgroups. The committee is to consist of Dr. Philip Levine, Raritan, N. J., chairman; Dr. R. R. Race, London, England; Dr. J. M. Hill, Dallas, Texas; Dr. Bruce Chown, Winnipeg, Canada; Dr. E. Uribe Guerola, Mexico, D. F.; Dr. Israel Davidsohn, Chicago, Illinois; and Dr. Sol Haberman, Dallas, Texas.

4. A routine Rh testing for all transfusions and pregnancies was recommended. Since Rh serum is not available on a world-wide basis, the following recommendations were made: When serum is not available in large quantities, only women should be routinely tested for the Rh factor. Anti-D serum having 85 per cent specificity, or anti-CD serum having 87 per cent specificity should be used. If the population or race of people concerned, only rarely exhibits Rh negative individuals, routine testing may be restricted to the female sex. If, however, the group has the normal, or approximately normal, distribution of individuals who are Rh negative, routine testing of both sexes must be done.

5. Chown's capillary test was recommended for routine Rh testing when testing serum with saline agglutinins. If a nonsaline agglutinating serum is all that is available, the Diamond test, using the slide method, or, in suitable cases, the Chown capillary tube test, are highly recommended. These technics, however, are to be left for review by the committee.

6. The compatibility test of Witebsky for use as the crossmatch in blood banking was highly recommended, but not adopted.

7. Dr. J. M. Hill of Dallas, Texas, announced that Baylor Hospital, and the William Buchanan Blood Bank and Serum Center, have offered their facilities in a cooperative project for the production and standardization of serum. This organization is now operated on a non-profit basis with all income in excess of cost going to research. It is contemplated that the funds received from this project may be used in part to finance and support an international organization for the study of blood. The committee appointed to study

this matter is composed of Dr. J. M. Hill, chairman, Dallas Texas; Dr. Louis K. Diamond, Boston, Mass.; Dr. W. S. Stanbury, Toronto, Canada; Dr. Luis Gutierrez Villegas, Mexico City; Dr. Ernest Witebsky, Buffalo, N. Y.; and Dr. A. E. Mourant, London, England.

The joint meetings held in Dallas and Mexico City were successful in bringing together investigators from five countries and resulted in a free exchange of information and ideas. The hospitality extended in both cities made the meeting most enjoyable, while the international tone of the sessions fostered good will and understanding. The formation of an international society for hematology seems desirable.

SOL HABERMAN, PH.D., SECRETARY
International Hematology and Rh Conference

TEXAS SOCIETY OF PATHOLOGISTS

The Texas Society of Pathologists held its annual meeting at Houston, Texas, January 26, 1947. The morning was devoted to a business session and, in the afternoon, a seminar on microscopic pathology was held at Baylor University College of Medicine. Dr. S. A. Wallace, of Houston, conducted the seminar.

Officers of the Society for 1947 are: Dr. D. A. Todd, San Antonio, president; Dr. W. W. Coulter, Houston, president-elect; Dr. John F. Pilcher, Corpus Christi, vice president; and Dr. C. T. Ashworth, Dallas, secretary-treasurer.

The next meeting of the Society is scheduled to be held January 25, 1948, at Galveston, Texas.

AMERICAN SOCIETY FOR THE STUDY OF STERILITY

The third annual convention of the American Society for the Study of Sterility will be held at the Hotel Strand, Atlantic City, New Jersey, June 7 and 8, 1947. The convention will include original papers, round table discussions, scientific exhibits and demonstrations. Registration for the sessions is open to members of the medical and allied professions. Additional information may be obtained from the secretary, Dr. John O. Haman, 490 Post Street, San Francisco 2, California.

TECHNICAL SECTION

STABILIZED ANTIGEN SUSPENSION FOR USE IN THE KAHN TEST*

M. B. KURTZ, D.V.M., AND E. M. HILL, M.S.

From the Bureau of Laboratories, Michigan Department of Health, Lansing, Michigan

One of the limitations of the Kahn test for syphilis² is that the standard antigen when mixed for use in the test can be used for a period of only twenty minutes. When large numbers of serums are tested, it becomes necessary to prepare new suspensions at frequent intervals. If only a few serums are tested, antigen suspension is wasted, since all of each mixture may not be used. This report concerns a stabilized antigen suspension that can be used in the Kahn test for a period of at least thirty-six hours.

Kline⁴ and others^{1,6} use antigen suspensions in serologic tests which are satisfactory for comparatively long periods of time. Kahn,³ in an early article, mentioned an antigen suspension in which the lipins were resuspended in saline, but he made no mention of the length of time it was usable and did not incorporate this feature in his test. Recently, Price⁷ described a suspension, similar in principle to the one described below, which he stated could be used for at least six weeks.

The stabilized Kahn antigen suspension described here, can be prepared in sufficient quantity for at least a complete day's work and, most important, the suspension can be checked satisfactorily before use, thus assuring a uniform sensitivity and specificity throughout the day. Excess suspension is not wasted since the remainder can be used the following morning.

The stabilized antigen suspensions are prepared by mixing Kahn standard antigen with 0.9 per cent sodium chloride solution as in the Kahn technic. They are permitted to "age" for ten minutes. Following the interval of standing, they are centrifuged for three minutes at about 1800 R.P.M. The supernatant fluid is decanted and the precipitated lipins are resuspended in a volume of saline equal to the combined amounts of antigen and saline used in preparing the original suspension. Thus, if 1.4 ml. of saline was added to 1 ml. of antigen, the amount of saline for the suspension would be 2.4 ml. The resuspension constitutes the stabilized antigen and is ready for immediate use. If it is to be used the following day, it should be stored in the dark in a refrigerator.

The preparation of stabilized suspension with the Kahn presumptive antigen requires special attention in that presumptive antigen prepared in the usual manner is so highly dispersed that it cannot be satisfactorily sedimented by centrifugation. This difficulty is eliminated by using 1.5 per cent sodium chloride solution in the preparation of the original suspension instead of 0.9 per cent.

*Received for publication, February 1, 1947.

Otherwise, the preparation is identical with the method for preparing stabilized Kahn standard antigen suspension.

Stabilized suspensions give reactions slightly greater than those of the original suspension from which they are prepared. Therefore, should one desire to have the same level of sensitivity, it is necessary to use an antigen which has a slightly lowered sensitivity.

The stabilized standard antigen has been used officially in three series of examinations,^{5,9,10} and unofficially in one series,⁸ all sponsored by the United States Public Health Service for the evaluation of serodiagnostic tests for syphilis. The stabilized presumptive antigen was entered in one of the series of evaluation tests. A total of 3350 specimens from persons who had been examined by nationally known syphilographers was examined in the four series. Table 1 shows

TABLE 1
DATA COMPILED FROM PUBLICATIONS OF U. S. PUBLIC HEALTH SERVICE^{5, 8, 9, 10}

ANTIGENS USED	NUMBER OF SERIES OF EVALUATIONS OF SERODIAGNOSTIC TESTS FOR SYPHILIS CONDUCTED BY U. S. PUBLIC HEALTH SERVICE									
	A		B		C		D		E	
	Per cent		Per cent		Per cent		Per cent		Per cent	
	Sensi- tivity	Speci- ficity	Sensi- tivity	Speci- ficity	Sensi- tivity	Speci- ficity	Sensi- tivity	Speci- ficity	Sensi- tivity	Speci- ficity
Standard Kahn stabilized . .	68	100	81.7	100	65.7	100	66.6	99.8		
Standard Kahn.....			79.7	100	62.2	100				
Standard, in Kahn's lab- oratory	69	100	85.5	100	70.9	100	62.2	100		
Presumptive Kahn stabil- ized.....									79.4	98
Presumptive, in Kahn's laboratory									74.5	99.8
Total number of speci- mens tested	415	597	200	100	220	112	374	453	375	454

three types of comparisons: comparison of standard stabilized antigen with the standard procedure in our laboratory, and with the standard procedure in Dr. Kahn's laboratory, in two series; comparison of standard stabilized antigen in our laboratory with the standard procedure in Dr. Kahn's laboratory in two series; and comparison of presumptive stabilized antigen in our laboratory with the presumptive procedure in Dr. Kahn's laboratory in one series.

In addition, the reproducibility of results with portions of ten specimens of blood serum repeated daily over a period of ten days was checked in an evaluation series conducted by the United States Public Health Service.¹⁰ The curves in Figures 1 and 2 represent the results with the stabilized antigens. The results compare favorably with those obtained with the Kahn procedures.

The stabilized standard antigen suspension also has been used in routine tests

in comparison with the standard procedure in 2540 serums. The sensitivity was approximately two per cent greater with the stabilized antigen suspension. Satis-

REPRODUCIBILITY OF RESULTS WITH STANDARD KAHN AND STABILIZED STANDARD KAHN ANTIGENS

DILUTIONS*	NUMBER OF DAYS BETWEEN PREPARATION AND TESTING OF BLOOD SERUMS									
	5	6	7	8	9	11	12	13	14	15
3:4										
1:3										
1:4										
1:7										
1:9										
1:13										
1:25										
1:30										
1:44										
Neg.										

----- Standard by Dr. Kahn

—— Stabilized standard Kahn antigen

FIG. 1. The data in this figure are reprinted from *Venereal Disease Information*.¹⁰

* Dilutions of positive with negative specimens of serum.

REPRODUCIBILITY OF RESULTS WITH PRESUMPTIVE KAHN AND STABILIZED PRESUMPTIVE KAHN ANTIGENS

DILUTIONS*	NUMBER OF DAYS BETWEEN PREPARATION AND TESTING OF BLOOD SERUMS									
	5	6	7	8	9	11	12	13	14	15
3:4										
1:3										
1:4										
1:7										
1:9										
1:13										
1:25										
1:30										
1:44										
Neg.										

----- Presumptive by Dr. Kahn

—— Stabilized presumptive Kahn antigen

FIG. 2. The data in this are reprinted from *Venereal Disease Information*.¹⁰

* Dilutions of positive with negative specimens of serum.

factory comparisons of specificity were not made since clinical histories were not available. Testing of this stabilized antigen in other laboratories would be helpful in evaluating its usefulness in routine work.

SUMMARY

Antigen suspensions which can be used for at least thirty-six hours in the Kahn test have been investigated and have been found to compare favorably in sensitivity, specificity and reproducibility with the Kahn standard and presumptive procedures and to have definite technical advantages.

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ESTIMATION OF PROTHROMBIN LEVEL FROM PROTHROMBIN TIME*

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Numerous modifications^{1, 2, 4, 9, 10, 11, 20, 21} of Quick's original technic^{12, 13, 14} for the determination of plasma prothrombin have appeared in the literature. Each modification has been proposed because of a desire for increased accuracy, better reproducibility, or ease of technic. Recently, observers have stated that an increase in plasma prothrombin occurs in the presence of thrombo-embolic disease^{2, 3, 5, 18, 19} and after administration of certain drugs.^{7, 8} The increase can be detected only upon dilution of the sample to decelerate the speed of the recalcified clotting time. It occurred to us that some of the proposed modifications might not be accurate and we, therefore, re-examined the problem with the idea of obtaining a reliable method upon which to base further studies of thrombo-embolic disease.

Quick¹² showed that the concentration of prothrombin varied with the coagulation time of plasma in a hyperbolic manner. By the use of an optimal excess of calcium ion, a maximum of thromboplastin, and a water bath at 37 C., the coagulation time of oxalated plasma became dependent upon the prothrombin concentration. In this study, Quick's original calcium concentration of 0.025 M calcium chloride was used and was not varied because it appeared to be optimal.¹³

EXPERIMENTAL PROCEDURES AND RESULTS

Diluent

In order to translate the clotting time to terms of prothrombin activity, the clotting times of known concentrations of prothrombin, or of normal plasma, must be found. The known concentrations are prepared by diluting pooled normal plasma with a suitable diluent, preferably plasma from which only prothrombin has been removed.

Successive dilutions of pooled plasma from ten healthy young adults, diluted with identical plasma treated with barium sulfate, yielded clotting times shown in Table 1. A curve (Fig. 1) drawn from these results indicates a good hyperbolic relationship between percentage of plasma dilutions and clotting times. Clotting times can be observed with remarkable abruptness down to 1 per cent of whole plasma concentration. The clots are firmer than those obtained with aluminum hydroxide-treated plasma as the diluent. Comparison with the curve produced by aluminum hydroxide-treated plasma (Quick) shows that barium sulfate-treated plasma yields more rapid clotting times through the significant range of from 20 to 5 per cent (Fig. 1).

Barium sulfate appears to be a specific absorbant of prothrombin.^{6, 21} Oxa-

* Received for publication, January 8, 1947.

lated plasma treated with barium sulfate fails to clot at all when calcium and thromboplastin are added. Similar plasma treated with aluminum hydroxide,

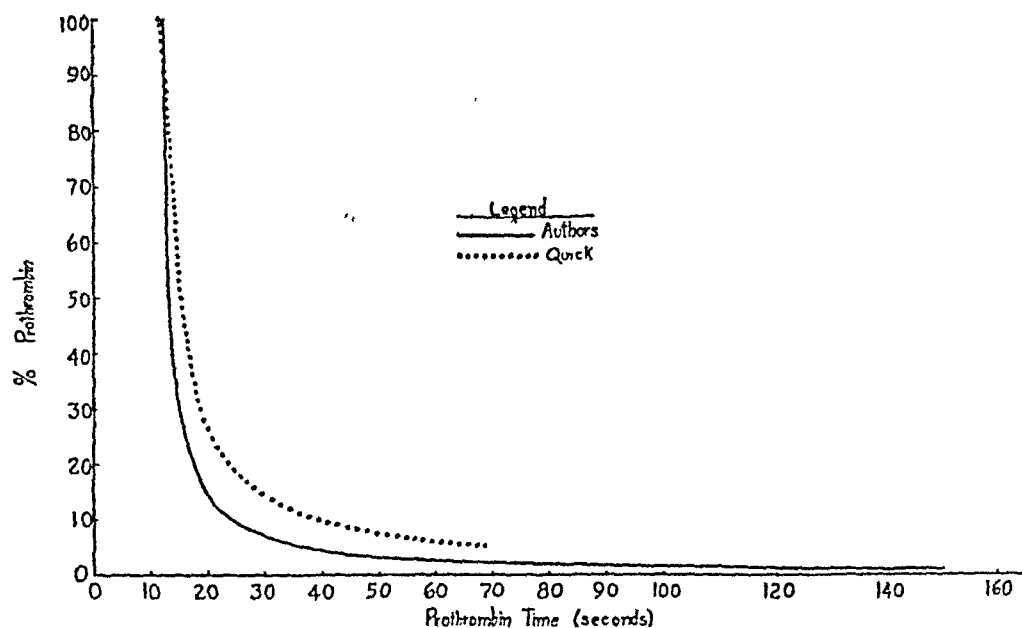


FIG. 1

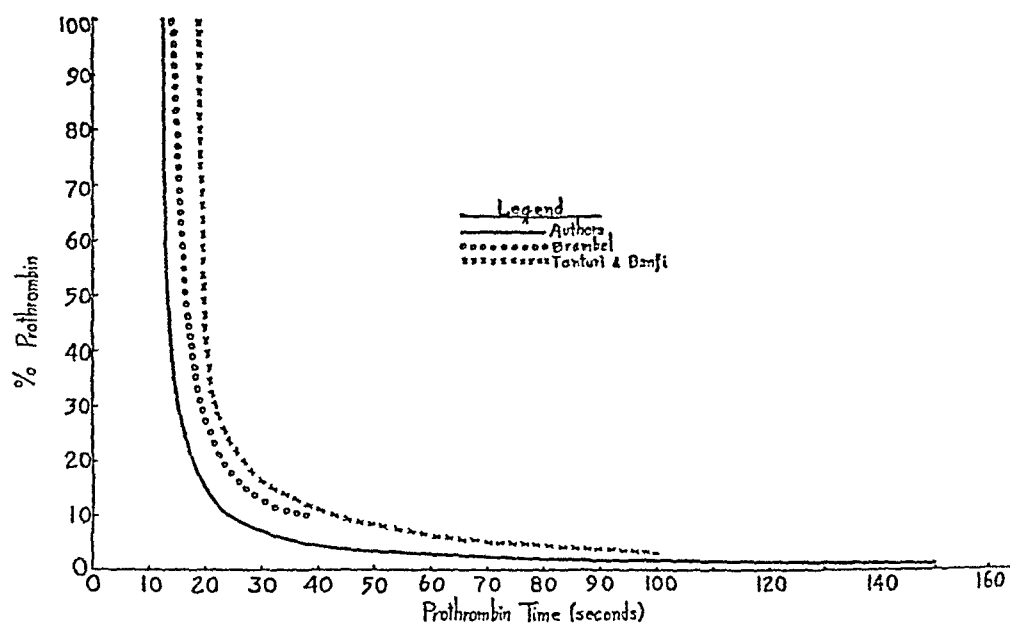


FIG. 2

as used by Quick,¹² however, does clot in from two to twelve hours when calcium and thromboplastin are added.

Barium sulfate removes approximately 80 to 100 mg. per cent protein material

from oxalated plasma as determined by Kjeldahl analysis (using 6.25 N factor). This figure is not particularly close to the suggested level of prothrombin as derived by chemical fractionation.¹⁷ The source of the extra protein is not clear at the present time but it can be stated that it is not fibrinogen. Barium sulfate removes no significant quantities of fibrinogen from plasma and aluminum hydroxide removes about 10 per cent (Table 2). Fibrinogen levels are determined by thrombin conversion and micro-Kjeldahl measurement of the resulting fibrin. Correction for the dilution of plasma which results from the addition of 30 per cent aqueous suspension of barium sulfate or of commercial aluminum hydroxide

TABLE 1

COMPARISON OF PROTHROMBIN TIMES OF VARIOUS DILUTIONS OF POOLED PLASMA PREPARED WITH BARIUM SULFATE-TREATED PLASMA AND ALUMINUM HYDROXIDE GEL-TREATED PLASMA

DILUTION OF PLASMA IN PER CENT	CLOTTING TIMES IN SECONDS, USING BaSO_4 -TREATED PLASMA AS DILUENT	CLOTTING TIMES IN SECONDS, USING $\text{Al}(\text{OH})_3$ -TREATED PLASMA (BY QUICK ¹²) AS DILUENT
Whole plasma (no diluent used)	12.4	12
50	13.3	16
30	15.1	21
25	16.6	23
20	18.1	26
15	19.6	31
12.5	20.7	
11	23.3	
10	23.9	39
9	25.6	
7.5	29.3	
6	33.3	
5	36.4	70
4	42.4	Curve not carried below 5 per cent
3	56.8	
2	74.0	
1	150.0	

is made by centrifuging the mixtures in hematocrit tubes and measuring the actual supernatant. The ratio of this to the original plasma content of the mixtures is the correction factor. For barium sulfate, the factor is 1.4 for the addition of 0.2 volumes; for aluminum hydroxide, it is 1.05 for the addition of 0.1 volume.

The use of saline as a diluent, as suggested by Brambel and others,^{1, 4, 20} was also studied. The results are extremely variable, and the end-points are not at all sharp. The method is also open to criticism because fibrinogen is diluted along with prothrombin, and the entire plasma protein balance is disturbed.¹⁴ A saline solution of fibrinogen was substituted for barium sulfate-treated plasma as a diluent. The pH and calcium ion concentration are difficult to control, but,

when carefully adjusted, the clotting times are practically identical with those found with barium sulfate-treated plasma (Table 3).

Thromboplastin

There have been many suggested modifications of the original thromboplastin reagent devised by Quick. Brambel¹ suggested a similar, but less active preparation, presumably by retaining small quantities of heparin, which he hoped would allow greater accuracy. Tanturi and Banfi²¹ proposed a very dilute suspension of the desiccated rabbit brain. Since these reports represented extremes in modifications, it was decided to compare them with Quick's thromboplastin on

TABLE 2
EFFECT OF BARIUM SULFATE AND ALUMINUM HYDROXIDE GEL ON PLASMA
FIBRINOGEN LEVEL

Grams of fibrinogen present in 100 cc. plasma	
In whole plasma.....	0.33
In supernatant from plasma treated with BaSO ₄	0.29
Corrected for dilution (×1.4).....	0.33
In supernatant from plasma treated with Al (OH) ₃	0.27
Corrected for dilution (×1.05).....	0.29

TABLE 3
COMPARISON OF PROTHROMBIN TIMES OF PLASMA DILUTED TO 10 PER CENT WITH
FIBRINOGEN SOLUTION AND BARIUM-TREATED PLASMA

DILUENT USED	SAMPLE 1				SAMPLE 2				SAMPLE 3			
	Tests			Average	Tests			Average	Tests			Average
	1	2	3		1	2	3		1	2	3	
Barium sulfate - treated plasma.....	20.5	19.6	19.9	20.0	18.9	18.9	18.7	18.8	27.0	27.3	27.6	27.3
Fibrinogen solution.....	21.3	21.2	21.5	21.3	20.0	19.3	19.3	19.5	27.8	28.0	28.3	28.0

successive dilutions of plasma with barium sulfate-treated plasma. The results are shown in Figure 2 and reveal Quick's thromboplastin to be the most active preparation. The structure of all three curves is remarkably similar, so that the significance of clotting times in the lower dilutions is obviously not enhanced by the use of a less active preparation of thromboplastin. Furthermore, the end points are much more difficult to observe with the less active preparations of thromboplastin.

Powdered thromboplastin prepared by Quick's method has been found to be stable, and the results obtained when fresh extractions are made daily are readily reproducible. This differs from the opinions expressed by Hurn, Barker and Magath.⁹ It has not been found necessary to standardize each batch of rabbit brain inasmuch as a fairly large amount of material is prepared at one time from

at least ten rabbits. The material keeps well in a vacuum desiccator in a standard refrigerator.

DISCUSSION

Emphasis on standardization of reports of prothrombin concentration from various laboratories has lead to a general agreement that the results should be reported in per cent of average normal, based upon a dilution curve.^{1, 9, 13} Because of the effect of the diluent, the validity of the curve used must be supported. From Figure 1, it can be observed that the thromboplastin was not a factor in the reading of per cent concentration, because the same material was used for both curves, and yet the indicated prothrombin concentration on Quick's curve at a given clotting time in the higher dilutions is almost double that found on the barium sulfate curve. If the barium curve shown in Figure 1 is acceptable as a valid index of prothrombin concentration, then arbitrary hemorrhagic levels quoted as 10 per cent after Dicumarol therapy will have to be revised. Prothrombin times equivalent to 10 per cent prothrombin concentration on Quick's curve, indicate an actual level of 5 per cent on the barium curve, and the hemorrhagic level of Dicumarol therapy should be quoted as 5 per cent. It is suggested that the question of the hemorrhagic level of Dicumarol therapy be revised in the light of these observations.

Regardless of which curve is adopted for use, variations in prothrombin concentration between 100 per cent and 30 per cent produce very little change in clotting time, and the significance on whole plasma of clotting times which are nearly normal is greatly reduced. When the plasma is diluted to 10 per cent, however, very slight changes in prothrombin concentration result in large changes in the clotting time. Based upon this observation, we have adopted a routine 10 per cent dilution with barium sulfate-treated plasma, in addition to the usual determination of prothrombin time on whole plasma.

TECHNIC OF METHOD

Reagents

- (1) 0.025 M calcium chloride
- (2) 0.1 M potassium oxalate
- (3) Quick thromboplastin solution. Mix 0.2 gm. of dehydrated rabbit brain with 5.0 cc. of fresh saline. Incubate at 45 to 50 C. for fifteen minutes, mixing frequently. Centrifuge at 200 to 400 R.P.M. for thirty seconds and remove the milky supernatant fluid. (Dehydrated rabbit brain is prepared by stripping the vessels and pia from the brains of several freshly killed rabbits, macerating the brains under several changes of acetone in a mortar until a nonadhesive powder is obtained, and drying on a suction filter. The preparation of brain keeps for at least six months in a large vacuum desiccator in a standard refrigerator at 4 C.).
- (4) Thromboplastin-calcium mixture, prepared by mixing equal quantities of (1) and (3).

Procedure

If fewer than three patients are to be tested in one run, draw 9.0 cc. of venous blood from each patient into chilled test tubes containing 1.0 cc. of 0.1 M potassium oxalate; if more

than three patients are to be tested, only 4.5 cc. of blood need be drawn into chilled tubes containing 0.5 cc. of oxalate. Mix by gentle inversion and chill at 4 C. until ready. Centrifuge the oxalated blood samples and remove as much supernatant plasma as possible. With a 1 cc. serologic pipet, transfer 0.1 cc. of plasma from each sample into four 10 mm. test tubes. Pool the remaining plasma and refrigerate until ready. Refrigerate all 0.1 cc. portions of plasma until actually ready to begin testing. Warm one tube at a time in a water bath at 37 C. for thirty seconds and add 0.2 cc. of previously warmed thromboplastin-calcium mixture, being careful to avoid foaming. Start timing with a stop-watch immediately and gently tilt the test tube in and out of the water bath until a clot is seen to form. A strong light behind the water bath facilitates observation. Three such 0.1 cc. portions are tested and the clotting times averaged as the prothrombin time of whole plasma. On a chart, such as Figure 1, read the per cent prothrombin corresponding to the observed prothrombin time.

To the pooled remaining plasma, add 0.1 gm. of barium sulfate powder for each cc. of plasma (or 20 per cent additional volume of a 30 per cent barium sulfate suspen-

TABLE 4
PROTHROMBIN TIME IN SECONDS AND IN PERCENTAGE OF PROTHROMBIN, ACCORDING TO
CONCENTRATION OF PLASMA USED

PERCENTAGE CONCENTRATION OF PLASMA	PROTHROMBIN TIME		REMARKS
	Clotting Time in Seconds	Percentage of Prothrombin	
(1) 100 10	12.6 23.9	100 100	Average reading, 100 per cent
(2) 100 10	15.4 40.7	25 35	
(3) 100 10	14.0 25.1	40 95	Variation is too great, so accept 10 per cent reading.
(4) 100 10	52 150	3.5 1.0	

sion in water). Shake well and incubate at 37 C. for ten minutes. Centrifuge at 3000 R. P.M. for thirty minutes and pour off the supernatant plasma. To 0.1 cc. of this plasma, add 0.2 cc. of thromboplastin-calcium mixture to ascertain that no clotting takes place. To the remaining 0.1 cc. portion of untreated whole plasma from each specimen, add 0.9 cc. of barium sulfate-treated plasma. Mix carefully and transfer 0.1 cc. portions of these 10 per cent dilutions to 10 mm. tubes, and proceed as with whole plasma. From Figure 1 read per cent prothrombin from the average observed prothrombin time and multiply by ten.

Readings of fourteen seconds are not unusual on certain normal whole plasmas, but the 10 per cent dilution readings indicate the same activity as a similar normal plasma with a prothrombin time of twelve seconds. When the 10 per cent figure is normal, or shows a great variation from that of the whole plasma, the 10 per cent reading is accepted as the accurate reading. When 10 per cent dilution reduces the prothrombin in the diluted plasma to about the level of 1 per cent or less, it is advisable, since concentrations of less than 1 per cent are diffi-

cult to read, to accept the whole plasma determination as the significant result. When the plasma fibrinogen is markedly reduced, as in severe liver disease, it may be necessary to determine the prothrombin time of a 50 per cent dilution so that the barium sulfate-treated plasma may be used as a means of adding fibrinogen to the system. Four examples from our laboratory files are listed in Table 4.

SUMMARY AND CONCLUSIONS

Quick's prothrombin determination was reviewed from the standpoint of proposed modifications.

Barium sulfate was tried as a means of removing prothrombin from plasma to enable use of the latter as a diluent. The results of a dilution curve with plasma so prepared were compared with those of Quick who used aluminum hydroxide gel.

Use of barium sulfate-treated diluent is believed to be superior to that of aluminum hydroxide gel, and the curve produced with it is proposed as the most accurate method of measuring prothrombin activity by means of a simple one-stage technic.

Thromboplastin preparations were compared and Quick's original material was found to be the most active, the most stable and the most duplicable.

It is suggested that the indicated prothrombin concentration derived from the prothrombin time of whole plasma be routinely compared with that from 10 per cent plasma made by diluting with barium sulfate-treated plasma.

Revision of suggested danger levels of prothrombin under Dicumarol therapy is suggested for future study.

Acknowledgment. The technical help of Miss Rose Mary Teresi, M.T. (A.S.C.P.) is sincerely appreciated by the authors.

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PERMANENT STAIN FOR AMYLOID*

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It has long been recognized that the metachromatic reaction of crystal violet is an excellent way to demonstrate amyloid substance microscopically, but unfortunately, it has proved difficult to maintain this stain for any appreciable length of time. That is why so many authors have turned to Congo red which, despite the fact that it is unreliable and nonspecific, can be mounted in clarite (or balsam) and thus be kept indefinitely.

In the following method, the crystal violet stain is made permanent by mounting the section in a water-soluble synthetic resin known as Abopon.† Frozen or hydrated paraffin sections are taken from water and placed in the staining solution from five minutes to twenty-four hours, then rinsed in water and mounted in Abopon.

The staining solution, which is stable, is made by diluting 10 cc. of a saturated alcoholic solution of crystal violet with 300 cc. of distilled water to which has been added 1 cc. of hydrochloric acid. The incorporation of acid with the staining solution renders the latter self-differentiating. Since overstaining cannot take place, the length of time in the stain is not important. When it is suspected that the amount of amyloid might be small, as in hyalinized islets of Langerhans, thicker sections should be cut in order to render the color reaction readily visible.

The staining solution used here was proposed by Eden,¹ but he recommended staining the undeparaffinized sections first, and then, after dissolving the paraffin as quickly as possible with xylol, mounting in balsam. This method is capricious, depending as it does upon penetration by the aqueous staining solution of varying thicknesses of paraffin. Often parts of a section do not stain at all.

About three years ago, it occurred to the author to try this staining solution on slides brought down to water in the conventional fashion. The amyloid in these sections was found to give a striking metachromatic reaction which unfortunately disappeared as soon as ethyl, butyl or isopropyl alcohol was used for dehydration in preparation for mounting. Ordinary aqueous mounting mediums, while a little better, also made the reaction fade. Finally Abopon was tried and, not only has the purplish red color of the amyloid failed to become blue like the rest of the section, but the original color has been maintained in slides stained two years ago. Therefore, it may be considered that a permanent stain for amyloid has been found.

Furthermore, this method of staining amyloid distinguishes well between amyloid and other hyaline substances which stain blue in contrast to the purple of the amyloid.

The Abopon mounting medium, which is made by dissolving with the aid of

* Received for publication, February 14, 1947.

† This material may be obtained from Glyco Products Co., 148 Lafayette Street, New York, N. Y.

heat, 50 gm. of the substance in 25 cc. of distilled water, keeps indefinitely and requires no preservative. In common with other aqueous mediums, however, it does have the disadvantage of "bubbling" on drying. So far, no satisfactory way has been devised to get rid of the entrapped air bubbles except by pressure on the cover slip after the mount has been made. Since these bubbles do not hamper reading of the slides, this fault is not serious.

SUMMARY

Permanent staining of amyloid in both frozen and paraffin sections is gained by placing the section in an acidified solution of crystal violet and then mounting in an aqueous solution of a synthetic resin known as Abopon. This stain requires no differentiation and distinguishes between amyloid and other hyaline substances.

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A RAPID METHOD FOR THE DETERMINATION OF SALICYLATES IN SERUM OR PLASMA*

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With the widespread therapeutic use of salicylates in massive doses, especially in patients with rheumatic fever, it is desirable to control the blood salicylates to maintain effective dosages. This method is presented to enable even the smallest of clinical laboratories to determine salicylates in serum or plasma with minimum expenditure of time and equipment. If the determination is made in plasma, the latter should not contain more than 6 mg. of sodium oxalate (or its equivalent) per ml. The method is based on the color reaction of acidified ferric nitrate with serum, without removal of proteins or interfering substances. It is applicable for use with the photo-electric or the Klett type of colorimeter, or for visual estimation. It has been used successfully for more than two years in four different army laboratories. Salicylates, in this determination, represent the total of salicylic acid plus the metabolic products of salicylic acid that contain the salicyl group.

REAGENTS

1. Hydrated ferric nitrate ($\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$), 1 per cent in 0.07 normal nitric acid.
2. Diluted ferric nitrate. Dilute the above reagent 5 to 9 with water (5 parts of reagent plus 4 parts of water).
3. Nitric acid, 0.038 normal. Dilute 0.07 normal nitric acid 5 to 9 with water (5 parts of reagent plus 4 parts of water). Concentrated nitric acid (sp. gr. 1.42, 70.5 per cent), 4.69 ml. in water up to 1 liter produces a solution which is approximately 0.07 normal. Extreme accuracy is not essential in preparing the above reagents. The amounts given represent the optimum concentrations, but considerable variation is permissible.

PROCEDURE

In a suitable colorimetric tube, add 9.0 ml. of the diluted ferric nitrate reagent to 1.0 ml. of serum. In another similar tube, add 9.0 ml. of 0.038 normal nitric acid to 1.0 ml. of serum. With the wave length set at 530 $\text{mm}\mu$, adjust the colorimeter to 100 per cent transmission with the acidified serum and read the color intensity of the reacted serum. Read the salicylate level directly from a previously prepared graph. It may be read immediately or after several hours since the color is quite stable. Any photo-electric colorimeter may be used. If a spectrophotometer is used, the wave length should be set at 530 $\text{mm}\mu$. In a colorimeter using filters, the transmission of the filter should be approximately 530 $\text{mm}\mu$.

To prepare the graph, pool samples of serum from about ten normal persons who have not been taking any form of salicylates. Repeat the procedure out-

* Received for publication, November 27, 1946.

lined above using the pooled serums. The optical density obtained represents the intensity of the color that is formed when acidified ferric nitrate reacts with normal serum. This average value will be subtracted from the total optical density obtained in the determination. In terms of salicylic acid it amounts to about 4 or 5 mg. per 100 ml. at a wave length of 530 $m\mu$. Variation of any one normal serum from this average value rarely exceeds 1.5 mg. per 100 ml. in terms of salicylic acid.

A stock salicylate solution is made to contain 1.00 mg. salicylic acid or 1.16 mg. sodium salicylate per ml. From this stock solution, a 1 to 10 dilution is made which contains 0.1 mg. salicylic acid per ml. To seven colorimetric tubes, add 0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 ml., respectively, of the standard solution. Water is added to each tube to make 5.0 ml. of solution. These standards represent blood salicylate levels of 0, 5, 10, 20, 30, 40 and 50 mg. To each tube add 5.0 ml. of the 1 per cent ferric nitrate solution. Read each tube in the colorimeter using the first or blank tube to adjust the transmission to 100 per cent. To each value obtained, add the optical density of the interfering color found in the normal serum. These values are then plotted on rectangular graph paper against the corresponding blood levels and the graph is then ready for use. To prepare a graph for higher levels, use a stronger standard solution and proceed in the same manner as outlined.

If a photo-electric colorimeter is not available, a Klett type of visual colorimeter may be employed. The unknown is first allowed to react with the acidified ferric nitrate in order to obtain the approximate salicylate level. Use 1.0 ml. of serum plus 9.0 ml. of diluted reagent. Two or three standards are made which have approximately the same color intensity as the unknown sample. To make the standard, place 1.0 ml. serum which does not contain salicylates (preferably pooled serum) in a suitable colorimetric tube, add the necessary amount of standard solution, dilute to 5.0 ml. with water, and then add 5.0 ml. of the reagent. Select the standard that visually most nearly matches the unknown and match the unknown against it in the colorimeter. Calculation is made in the usual manner.

If no colorimeter is available, the unknown may be compared visually to a set of standards made as described above. The error by this estimation is less than 5 mg. per 100 ml. and results are satisfactory for clinical use.

The above procedures may be adapted for use with 0.5 ml. of serum, and may be more desirable for the analysis of specimens with high salicylate levels. Add 4.5 ml. water to 0.5 ml. of serum, followed by 5.0 ml. of the diluted ferric nitrate reagent. The blank consists of 0.5 ml. serum, 4.5 ml. water, and 5.0 ml. of the 0.038 normal nitric acid. A standard curve must be prepared accordingly.

To use the test for urine samples, add 0.5 ml. water and 9.0 ml. of the diluted ferric nitrate reagent to 0.5 ml. of the urine. Prepare standards using pooled normal urines containing no salicylates. Large quantities of salicyluric acid in the urine, however, invalidate the test except as an indication of the total salicyl output.¹

SUMMARY

A method for blood serum salicylates is presented which is simple and reliable. The recovery of salicylate is approximately 100 per cent. The method is applicable to visual estimation or to the Klett type of colorimeter, with only a slight loss of the accuracy obtained with photo-electric colorimeters.

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A QUANTITATIVE METHOD FOR DETERMINATION OF FRAGILITY OF ERYTHROCYTES*

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Conventional methods for determining fragility of erythrocytes as commonly used in clinical pathologic laboratories, provide a simple, practical means of determining relatively gross variations from normal. In experimental studies, however, in which precise quantitative data are desired, these methods are inadequate. Several technics have been described which enable quantitative determination of erythrocyte fragility.¹⁻⁵ The following method, however, incorporates one or more features which are not included in technics previously described, and provides a relatively simple means for the precise determination of the property of fragility as affected by varying concentrations of saline.

METHOD

Heparin is used as the anticoagulant in an amount sufficient to prevent clotting for one or two hours, in order to allow enough time to permit determination of the hematocrit reading and to make a saline suspension of the blood. One mg. of heparin per 20 ml. of blood of rats was found to be adequate.

A Wintrobe hematocrit tube, filled with heparinized blood, is centrifuged for thirty minutes at 3000 R.P.M. in an angle centrifuge. During the period of centrifugation, 15 serologic tubes are selected and labeled, and to each of 14 of the tubes, 1.3 ml. of sodium chloride solution is added. The solutions of sodium chloride added to these tubes are of different concentrations. The desired final concentrations are obtained by adding 0.3 ml. of a 10 per cent suspension of erythrocytes in isotonic saline to different initial concentrations of sodium chloride, as shown in Table 1.

The 1 per cent solution of sodium chloride is prepared in a liter volumetric flask using sodium chloride which has been thoroughly dried at 180 C. for sixteen hours. The initial concentrations are made, using a 100 cc. quantitative buret, preferably an automatic refill buret. On the basis of the hematocrit reading, a 10 per cent suspension of erythrocytes is obtained by adding the proper proportion of heparinized blood to 0.86 per cent sodium chloride solution. At least 5.5 ml. of this suspension should be prepared. Add 0.3 ml. of the 10 per cent suspension of erythrocytes to each of the 15 tubes and mix gently by inverting each tube three or four times. Stopper the tubes to prevent evaporation, and allow them to stand at room temperature for two hours, mixing gently by inverting them every fifteen minutes.

At the end of two hours, the tubes are centrifuged at 1500 R.P.M. (International centrifuge #2) for fifteen minutes. The centrifuge is allowed to stop

* Received for publication, February 14, 1947.

with the brushes lifted in order to minimize agitation and, thus, to avoid any resuspension of erythrocytes.

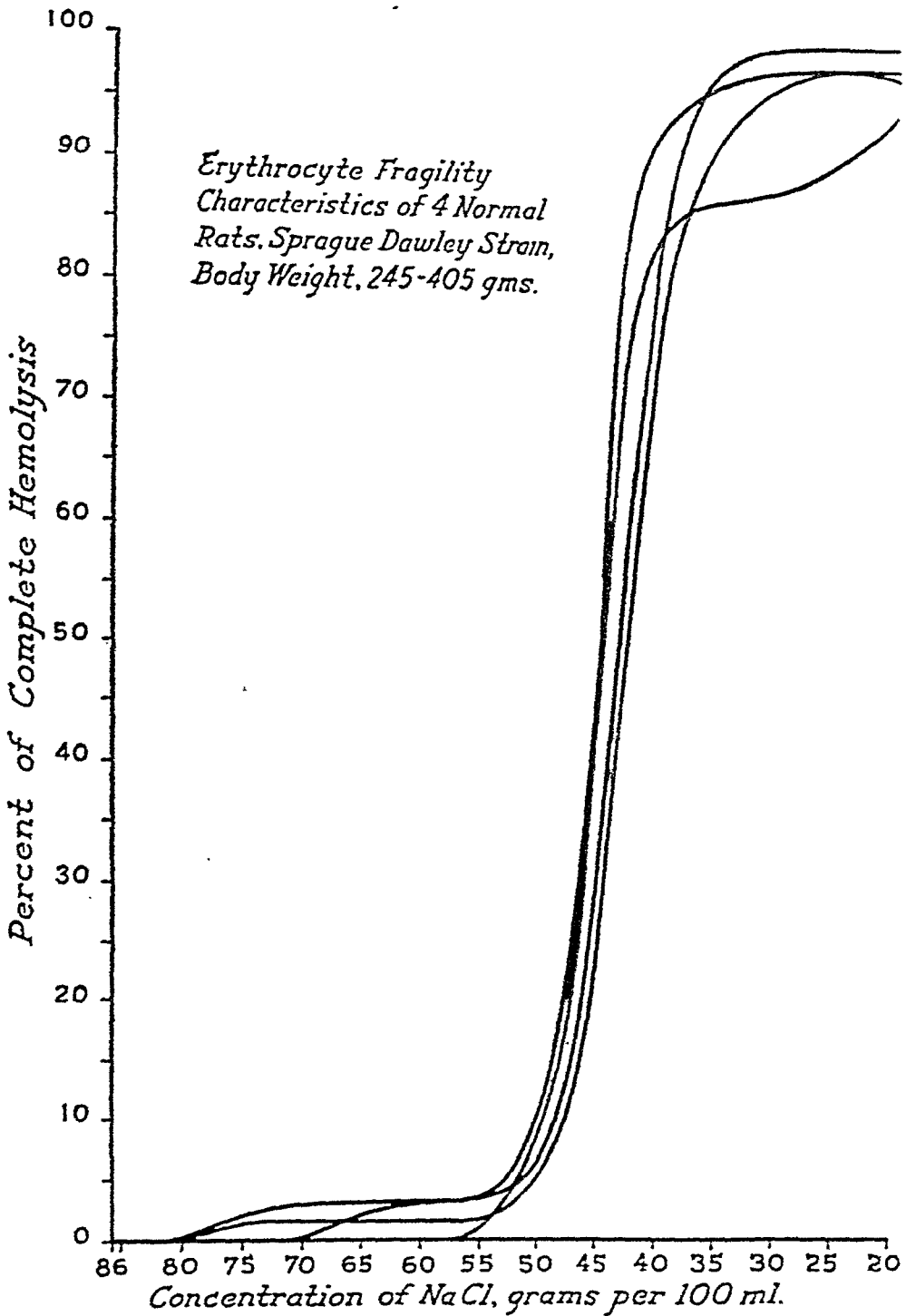


FIG. 1

Immediately following centrifugation, 0.5 ml. of the supernatant from each of the 15 tubes is added to macro-colorimetric tubes, each of which contains 4.5 ml.

of distilled water. Care must be taken not to include any erythrocytes with the supernatant removed. Following thorough mixture, the amount of hemoglobin is determined in a photo-electric colorimeter,* using a green filter (Klett #52). The supernatant from the 0.86 per cent solution of sodium chloride is used as a blank. The sodium carbonate solution (complete hemolysis) is used as the standard of 100 per cent hemolysis. Thus, each determination has its own blank and standard of 100 per cent hemolysis. From the data thus obtained, the degree of hemolysis can be accurately expressed in percentage of the total.

In Figure 1, data are presented obtained by the method described from a group of four normal male rats (Sprague Dawley strain). This is introduced for purposes of illustration and represents but a portion of data secured from a larger study, the results of which will be reported at another time.

TABLE 1

METHOD OF PREPARATION OF SOLUTIONS OF SODIUM CHLORIDE OF VARIOUS CONCENTRATIONS

Initial Preparation of NaCl Solutions														
Ml. 1% NaCl	6.20	12.33	18.37	24.41	30.45	36.47	42.52	48.56	54.60	60.64	66.68	72.72	78.75	86.00
Ml. distilled H ₂ O	93.71	87.67	81.63	75.59	69.55	63.53	57.48	51.44	45.40	39.36	33.32	27.28	21.25	14.00
1.3 ml. of the Above Solutions plus 0.3 ml. of 10% Erythrocytes in 0.86% NaCl Provides Solutions of NaCl of Following Designated Concentrations														
Tube number	1	2	3	4	5	6	7	8	9	10	11	12	13	14*
Percentage of NaCl in final solution	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.86
														1.3 ml. 0.10% Na ₂ CO ₃

* Tube 14 is used as the blank

† Tube 15, in which erythrocytes are completely hemolyzed, provides an individual standard of 100 per cent hemolysis.

SUMMARY

A method is described for precise quantitative determination of fragility of erythrocytes in which the following features are incorporated: the amount of erythrocytes is accurately determined on the basis of *volume* so as to compensate for variations in hematocrit reading and/or mean corpuscular volume; precise concentrations of sodium chloride solutions are used; the time of exposure of erythrocytes to saline solutions and the degree of mixture is standardized; the amount of hemolysis is determined objectively by means of a photo-electric colorimeter; each sample of blood is tested with its own standard of 100 per cent hemolysis and its own blank; and the results are expressed on the basis of per cent of total hemolysis.

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* A Klett-Summerson photo-electric colorimeter was used in these studies.

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A SIMPLE MICRO-PROJECTOR*

HAROLD GORDON, M.D.

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The success of clinicopathologic conferences depends, to a large extent, upon efficient demonstration of microscopic lesions. Many hospitals lack facilities for projecting microscopic slides, and the micro-projectors available commercially are none too satisfactory. Some are expensive, require much space and are difficult to operate; others give poor illumination or unsatisfactory magnification.

The projector illustrated below is an adaptation of a method used at many small hospitals. During the past seven months it has been used at this hospital at weekly clinicopathologic conferences, attended by thirty to forty staff men, residents and senior medical students. It is generally agreed that this projector is, at least, the equal of many of the more expensive projectors used at conferences elsewhere.

METHOD

A general view of the projector is shown in Figure 1. The source of light is a 300 watt lamp† combining a mercury vapor discharge and an incandescent tungsten electrode, operating on 11 volts and drawing 30 amperes. It operates on a transformer and gives excellent illumination while generating very little heat. The light is housed in a special unit manufactured by Bausch and Lomb Optical Company. It was designed for photomicrography and thus can be made to serve a dual purpose. The bulb has a high color temperature, will last for 150 hours or more and costs only \$10.50. The light is centered carefully through a microscope which is secured with a simple clamp.

A translucent screen made of tracing paper tightly stretched over a wooden frame is used which permits the operator to stand proximal to the screen, project the slides and point out lesions with a pencil. The audience, seated distal to the screen, sees the projected section and the sharp black shadow of the pointer (Fig. 2). A somewhat less efficient method of illumination can be improvised by using a Bell and Howell 16 mm. moving picture projector provided with a 110 volt, 60 cycle, 750 watt prefocused lamp. This also gives excellent illumination. Fairly satisfactory results can be secured by using a good microscope light, but this is not recommended, except for small groups.

SUMMARY

An inexpensive and efficient method for projecting microscopic sections to audiences of moderate size is described and illustrated.

* Published with permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or conclusions drawn by the author. Received for publication, January 9, 1947.

† Manufactured by the General Electric Company.

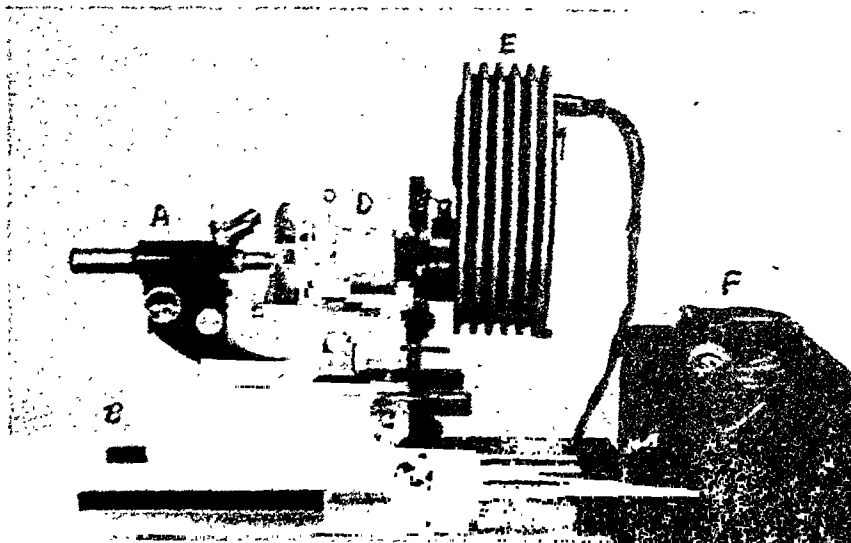


FIG. 1. GENERAL VIEW OF MICRO-PROJECTOR

Microscope (A) is clamped to wooden board (B) with clamp (C). The specially housed light (E) is directed through the substage condensor. A tin cylinder or can (D) prevents dispersion of light. (F) is a conventional transformer which allows the light to operate on 110 volt A. C. power. When in operation, a screen of black cloth is arranged around the entire unit in such a way as to prevent escape of any incident light.

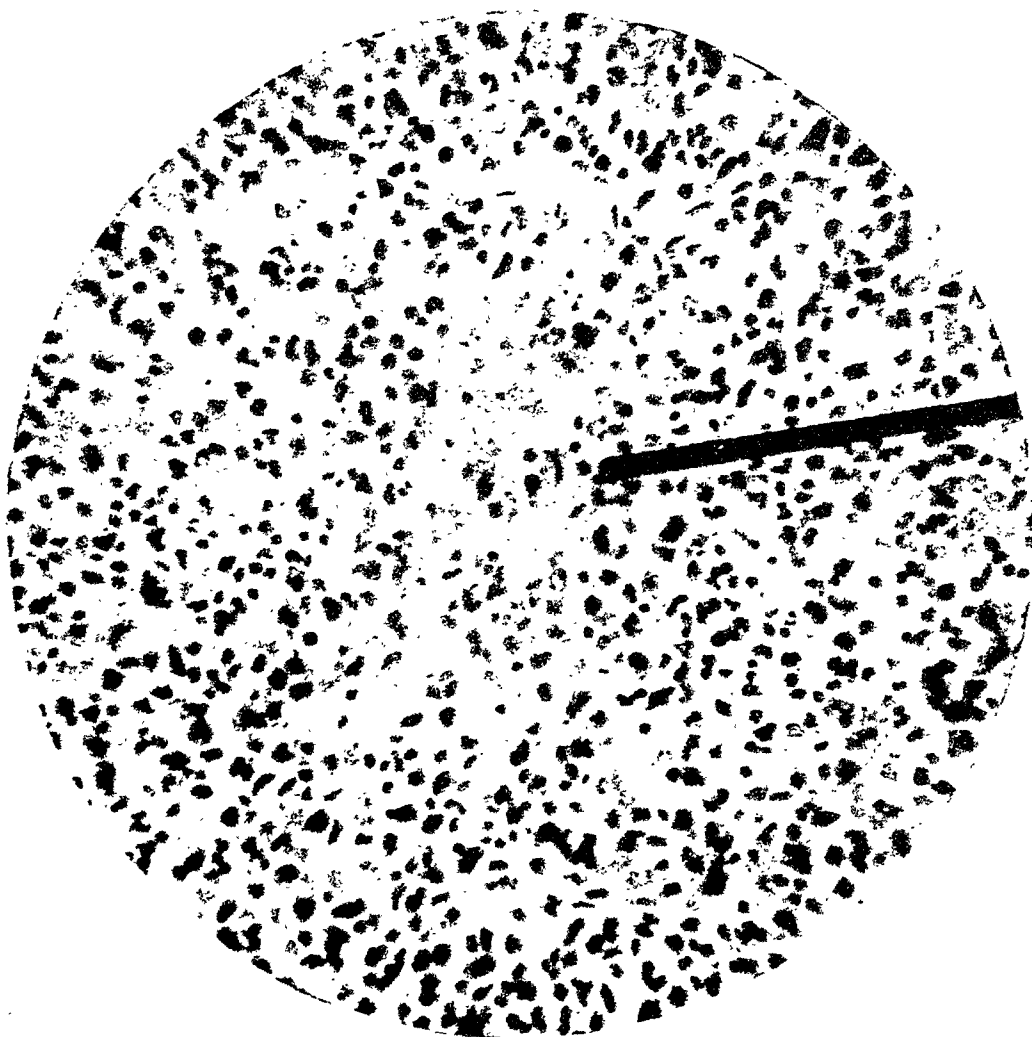


FIG. 2. PHOTOGRAPH OF A PROJECTED SECTION OF VERTEBRAL MARROW FROM A CASE OF PERNICIOUS ANEMIA

With the screen about 8 feet from the projector, using a 10 X eyepiece and a 16 mm. objective, a magnification of 125 diameters is obtained.

NEWS AND NOTICES

AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS

The Fifteenth Annual Convention of the American Society of Medical Technologists will be held in Denver, Colorado, from June 30 to July 2, 1947. Headquarters for the convention will be the Shirley Savoy Hotel. Miss Elizabeth O'Toole, 1345 Elati Street, Denver 4, Colorado, is in charge of local arrangements.

CANADIAN SOCIETY OF LABORATORY TECHNOLOGISTS

The tenth anniversary of the incorporation of the Canadian Society of Laboratory Technologists under Dominion Charter will be celebrated at the Society's convention, May 29, 30 and 31, 1947, at the Royal Connaught Hotel, Hamilton, Ontario, Canada. Papers, scientific movies and exhibits of various aspects of laboratory technic will be presented. Nonmembers are welcome at all but the business sessions. Information may be obtained from Miss Helen L. Smith, secretary, Canadian Society of Laboratory Technologists, 294 Barton Street East, Hamilton, Ontario.

TECHNICAL SUGGESTIONS

HOLDER FOR COUNTING PIPETS

In taking blood counts, if a special hematology tray is not available to hold the pipets, it is convenient to fasten the pipets to an ordinary slip of paper or to the request card of the

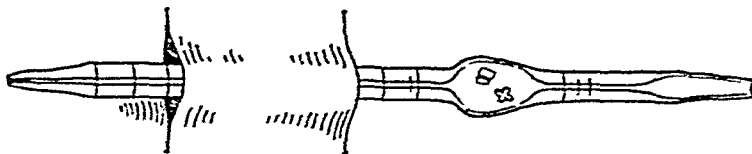


FIG. 1

patient. Two parallel cuts are made in the paper, each about three-fourths of an inch in length, one inch apart. The pipet is then inserted through the slits as shown in the accompanying figure.

E. MARIE SMITH, M.T. (A.S.C.P.)

AVOIDANCE OF IRRITATION TO LIP FROM COUNTING PIPETS

The technician engaged in the daily task of collecting numerous blood counts may find her lower lip becoming irritated and her teeth sensitive from constant pressure of the plastic mouthpiece used in this procedure. If the entire length of the mouthpiece is pushed back into the rubber tubing, the tubing will serve nicely as a cushion for the teeth and lips.

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STAINING OF YEASTS IN SPUTUM

An extremely good differential stain of yeasts and molds is obtained by the Papanicolaou staining method, in which Hematoxylin, Orange G and EA50 are utilized. In staining sputum, as for malignant cells, I have found that the leukocytes stain deep blue; erythrocytes, reddish orange; yeast spores, brown; their budding cells, red; and their filaments, reddish brown; while other bacteria have a blue shade different from that of the leukocytes.

ANNE KOFFINKE, M.T. (A.S.C.P.)
Grace Hospital
New Haven, Connecticut

HEMOGLOBINURIC NEPHROSIS IN TRAUMATIC SHOCK*

TRACY B. MALLORY,† LT. COL., MC, AUS

From the Fifteenth Medical General Laboratory, United States Army

Army experience has made many of us familiar with the syndrome of renal insufficiency following resuscitation from shock. The associated kidney lesion is constant but not specific. Lucké,¹⁴ in a study of material at the Army Institute of Pathology, has shown that a similar lesion, which he has termed "lower nephron nephrosis", may develop in a variety of conditions such as mismatched blood transfusions, the crush syndrome, blackwater fever, heat stroke, high altitude anoxia, thermal and chemical burns, carbon tetrachloride and mushroom poisoning, and sulfonamide sensitivity. I have seen it following anaphylactic shock, severe streptococcus infection and infantile gastro-enteritis, and it is possible to recognize many cases in the literature under such titles as interstitial nephritis¹² and hepato-renal syndrome.^{4, 11} There can be little doubt that with increasing breadth of experience many other predisposing conditions will be brought to light.

Descriptions of the lesion in various stages may be found in innumerable case reports, but surveys of any considerable mass of material are few, and no textbook of pathology contains an adequate description. An excellent description of the pathologic features may be found in the report of Bywaters and Dible⁶ based upon a series of cases of crush syndrome. My discussion is based primarily on a group of 60 fatal cases of battle injury, which were clinically and pathologically studied by members of a research team, whose full report will be published elsewhere. The observations on this limited group of cases are reinforced by a survey of over 200 similar cases from the files of the Fifteenth Medical General Laboratory. The importance of the lesion in military medicine is shown by an incidence of 18.6 per cent among 427 unselected autopsies on battle casualties dying in Army hospitals in Italy.

THE CLINICAL PICTURE

In a typical instance, a patient has been severely wounded and some hours have elapsed between injury and entrance to a field or an evacuation hospital. He has received some plasma en route, but is in a state of moderate to severe shock on arrival. The blood pressure is below 80 mm. systolic, possibly unmeasurable, the pulse rate 100 to 120 per minute, the color pale or ashen and the extremities cold. It is evident that there has been severe hemorrhage and a transfusion of whole blood is started as rapidly as possible. Two or three transfusions, perhaps more, probably will be needed before he can be taken to the x-ray department and the operating room. From twelve to twenty-four hours will elapse between

* Presented at the Twenty-Fourth Annual Meeting of the American Society of Clinical Pathologists, in San Francisco, June 29, 1946. Received for publication, March 31, 1947.

† Present address: Massachusetts General Hospital, Boston 14, Massachusetts.

injury and definitive treatment. During this period he has probably passed little urine, but no concern is felt. On the following day he is out of shock and his condition appears satisfactory. A measurement of urinary output, however, will show an oliguria, perhaps a total output of from 200 to 300 cc. for the day. On the third day the story is much the same. He may void spontaneously one or more times, but the twenty-four hour urine volume is below 500 cc. A non-protein nitrogen determination at this time would undoubtedly show nitrogen retention in the neighborhood of from 60 to 80 mg. per cent. If a blood pressure reading were to be taken on this day, it is probable that moderate elevation of both systolic and diastolic levels would be found, ranging from 140 to 180 mm. mercury systolic and from 90 to 100 mm. diastolic. As days pass, the hypertension and oliguria persist and urinary output may drop to "anuric" levels below 100 cc. *per diem*. The patient may be lethargic and drowsy, but is readily aroused and answers questions rationally. He seldom complains of headache, nausea or blurring of vision. Edema begins to appear and is likely to be predominantly pulmonary, often developing with paroxysmal suddenness and frequently serving as the immediate cause of death at any time from the third day on. If pulmonary edema is avoided, the nitrogen retention steadily mounts, lethargy deepens and eventually coma supervenes. Convulsive phenomena are unusual, but may appear. Uremic retinitis has been observed, but few ophthalmoscopic observations have been recorded. Pericarditis is very uncommon. Severe acidosis with Kussmaul breathing is seldom noted, perhaps because of the frequent use of intravenous alkali therapy. Once persistent oliguria and hypertension appear, the prognosis is extremely grave and the chance of survival is not much more than 20 per cent.

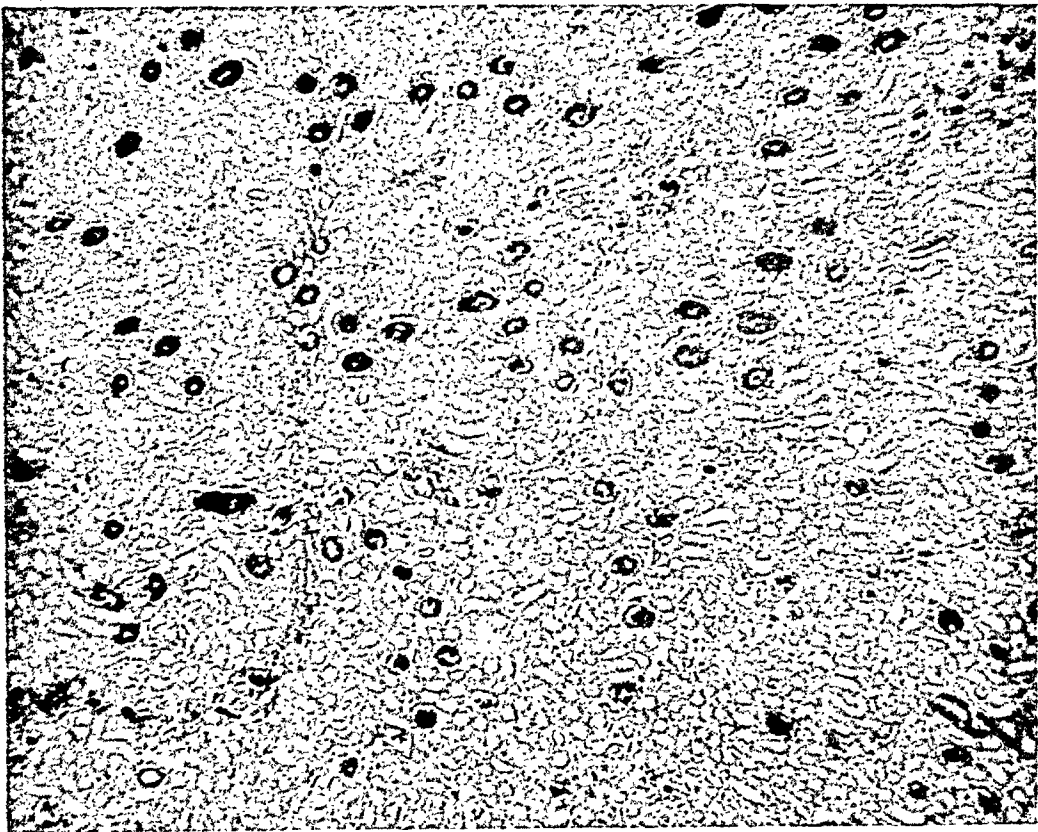
Such urine as is present is acid in reaction, low in specific gravity and contains albumin. Red cells and pigment granules may be found in the sediment and granular casts are usually present. Except in frank transfusion reactions and in crush cases, the urine is rarely "port-wine" in color. In the first or second specimen, even though the color is normal, a benzidine test after centrifugation will be positive, but subsequent specimens are often negative. Chemical determinations on the blood show, as would be expected, progressive rise in urea, creatinine and phosphorus, and decline in carbon dioxide combining power. In differentiating renal from prerenal oliguria and azotemia, rise in blood pressure and fixation of specific gravity proved to be our most reliable criteria.

THE RENAL LESIONS

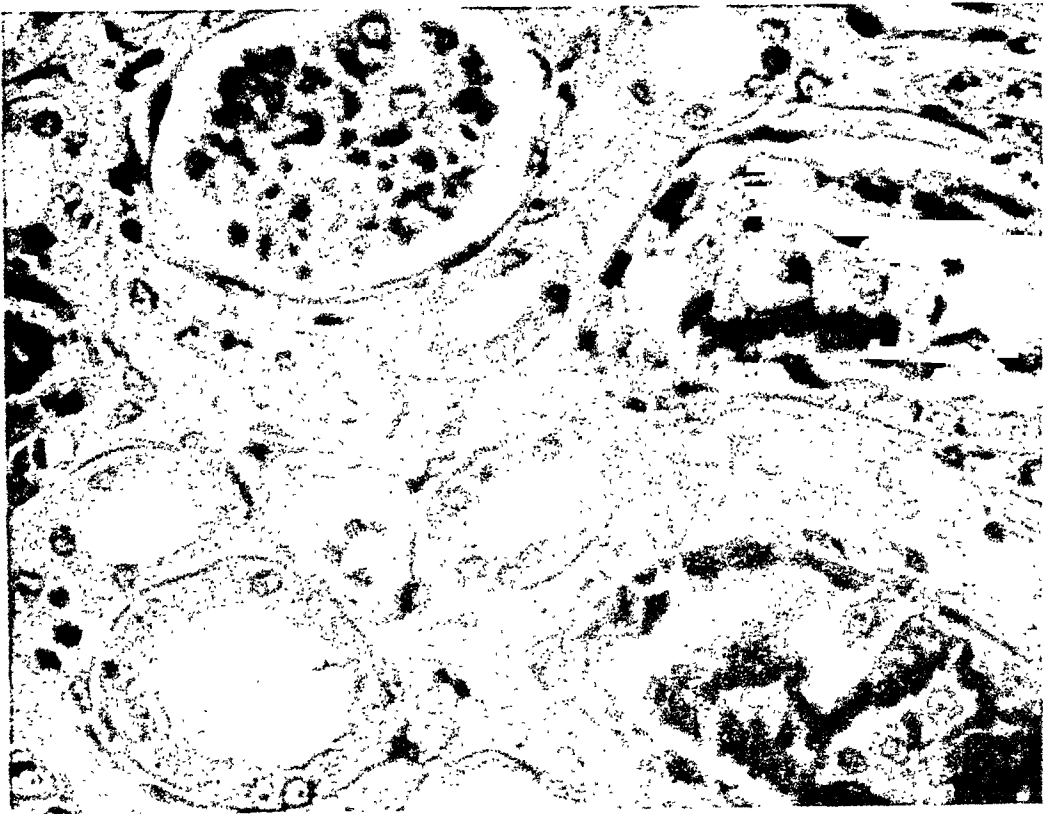
The kidneys are characteristically enlarged with a pale swollen cortex and deep purple pyramids. The cut surface is very moist. The kidneys may, however, show very little abnormality on gross examination. In the average case, the first and most obvious finding on microscopic examination is the presence of pigmented

FIG. 1. A portion of the medulla showing the majority of the collecting tubules plugged with pigment casts. Low power. Three-day lesion.

FIG. 2. Cortex, high power. Two distal tubules are plugged with precipitated pigment. Their epithelium shows degeneration and desquamation. The proximal convoluted tubules (lower left corner) are dilated but the epithelium is intact. Four-day lesion.



1



2

casts in the lower portions of the nephrons. These extend from the ducts of Bellini upward through the collecting and distal convoluted tubules (Figs. 1 and 2). In unstained sections, these casts are basically orange in color, varying sometimes towards green, sometimes towards brown. In sections stained with hematoxylin and eosin, they are reddish orange. Usually they can be demonstrated to be positive to benzidine. Staining reactions with Mallory's phosphotungstic acid-hematoxylin, and with Masson's trichrome stain are variable, freshly formed casts usually showing the staining reactions of hemoglobin, older casts failing to do so and accepting the counterstains. At no time does the material give a positive iron reaction. The depth of pigmentation varies considerably. Some casts appear to be composed entirely of strands of precipitated pigment, others show only pigment granules of varying size and density distributed through a matrix of colorless material.

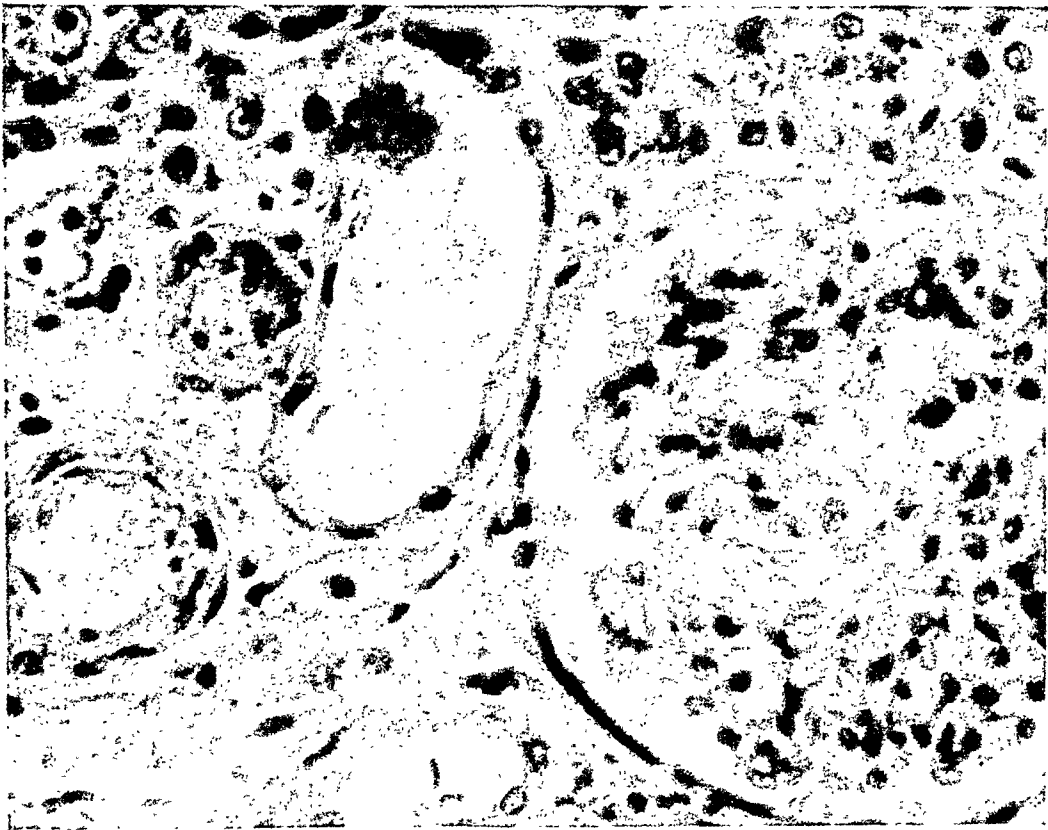
Less conspicuous is a second type of cast which is hyaline and quite colorless in the unstained specimen. It shows little affinity for either hematoxylin or eosin, but stains a bright green in the Masson preparations. These hyaline casts appear after the pigmented ones and are usually found at a higher level in the nephron, although mixed casts are not uncommon (Fig. 3).

Reactive changes in the epithelial cells of the lower nephron segment are a constant phenomenon. The first change is a fine fat vacuolization of the ascending limbs of Henle's loops. This appears in 75 or 80 per cent of shock cases regardless of whether clinical evidence of renal insufficiency develops or not, and I have presented, elsewhere, evidence that the process is reversible.² From the third day on, more severe degenerative changes are apparent in the same segment of the nephron. Frank necrosis and active regeneration appear (Fig. 4), herniations may develop and rupture of the tubule may occur (Fig. 5). In the collecting tubules, reaction is much less constant and appears limited to the immediate neighborhood of the pigmented casts. Reduplication of the lining cells, desquamation and active mitotic division may be present. In the tubules containing the nonpigmented casts, another type of degenerative change is found. The epithelial cells appear to disintegrate and the underlying basement membrane to disappear, permitting the cast to be extruded into the stroma (Figs. 6 and 7), a phenomenon to which we will return below.

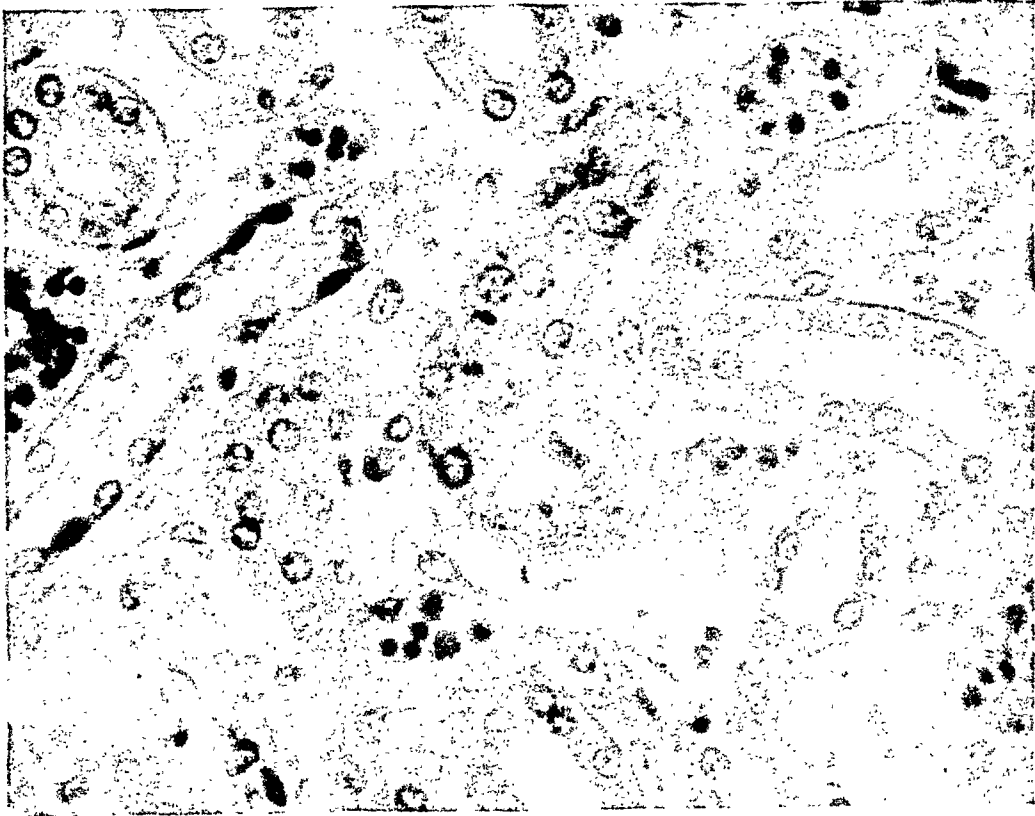
In contrast to these conspicuous changes in the lower portions of the nephrons, the proximal segments show little change. Glomerular tufts are invariably normal, although the epithelial cells of Bowman's capsule may be swollen, especially in crush cases. There has been much debate as to whether the proximal convoluted tubules are or are not dilated. An apparently marked dilation is usually seen in paraffin sections of formalin-fixed material. Comparison with Zenker-fixed material and the use of frozen sections on formalin-fixed tissue suggests that

FIG. 3. Cortex. A normal glomerulus and a dilated distal tubule with degenerative epithelial changes containing a mixed cast, chiefly hyaline but with a pigment mass at its upper pole. Five days.

FIG. 4. Straight tubules, probably ascending limbs near the cortico-medullary junction showing mitotic figure. A few lymphocytes are present in the stroma. Five days.



3



4

this is largely but not entirely an artefact, due to shrinkage. Probably slight to moderate dilation and some swelling of the epithelium are present in most instances, thus accounting for the gross widening of the cortex which is usually present in cases of five or more days' duration. The lumens contain much granular precipitate, probably albuminous in character. Necrosis of proximal tubules, however, has been seen in only 2 cases.

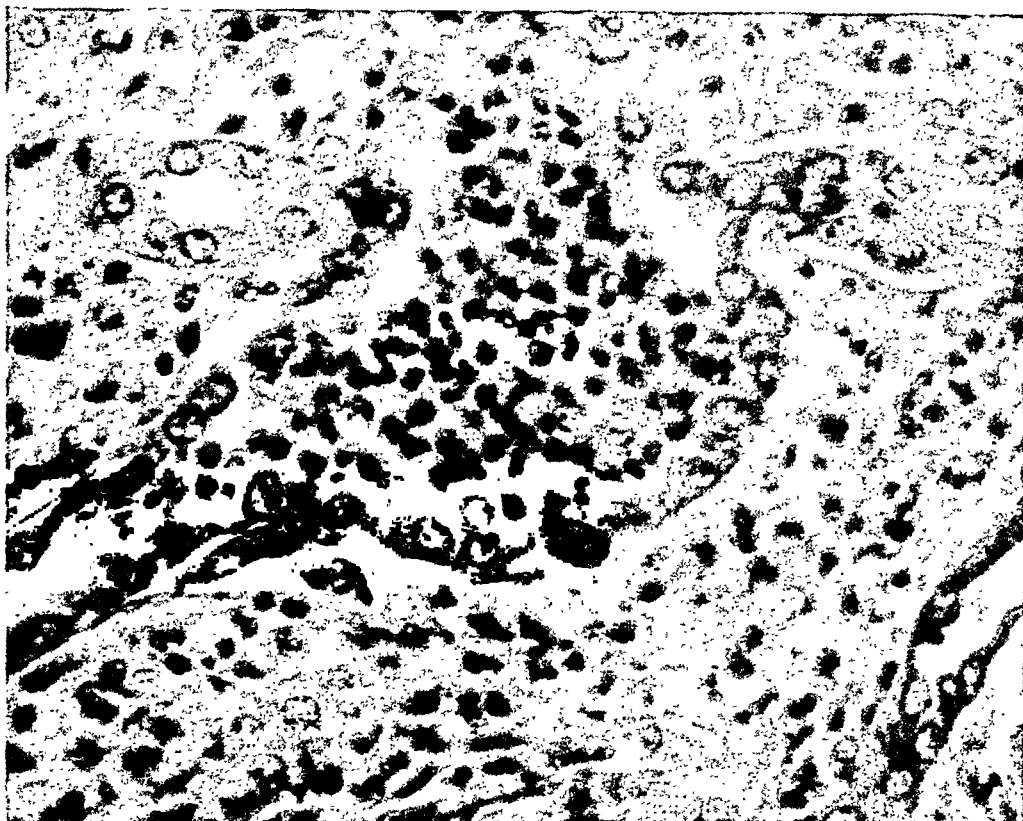
Reaction in these kidneys is not limited to the tubules. Beginning on the third day and well marked by the fourth, inflammatory infiltration appears in the stroma.* This is usually concentrated in a comparatively narrow zone, 1 to 2 mm. in width on the medullary side of the cortico-medullary junction, the same zone in which the degenerative tubular changes noted above tend to be most marked (Fig. 8). Occasionally, but infrequently, a second narrow inflammatory zone is found just beneath the capsule. There is also a definite tendency for the inflammatory changes to be emphasized in the immediate proximity of the blood vessels, particularly the veins. The inflammation is evidenced most constantly by lymphocytic and plasma cell infiltration (Fig. 9). Polymorphonuclears and eosinophils may accompany them in varying proportions but rarely predominate. Edema is not usually conspicuous, but may be discernible. If the inflammation is chronic (eight or more days), some fibroblastic proliferation may be seen.

A second type of inflammatory reaction usually, but not invariably, develops a day or two later than the one described above. This is granulomatous in character in that the predominating cell is histiocytic in type. It is always focal rather than diffuse. A majority of the granulomas form about masses of hyaline material identical in appearance and staining reactions with the hyaline intratubular casts already described. That at least a considerable portion of these masses represent extruded casts seems apparent from the frequency with which various stages of extrusion are observed. No other mechanism for their formation is suggested by any observations made to date. Once in the stroma, histiocytes collect about them in considerable numbers, first encapsulating and later invading the hyaline core. As time passes, this is broken up and partly absorbed (Figs. 10 and 11). In some granulomas no traces of it can be made out. Either it has been entirely absorbed or some of them develop by a different mechanism.

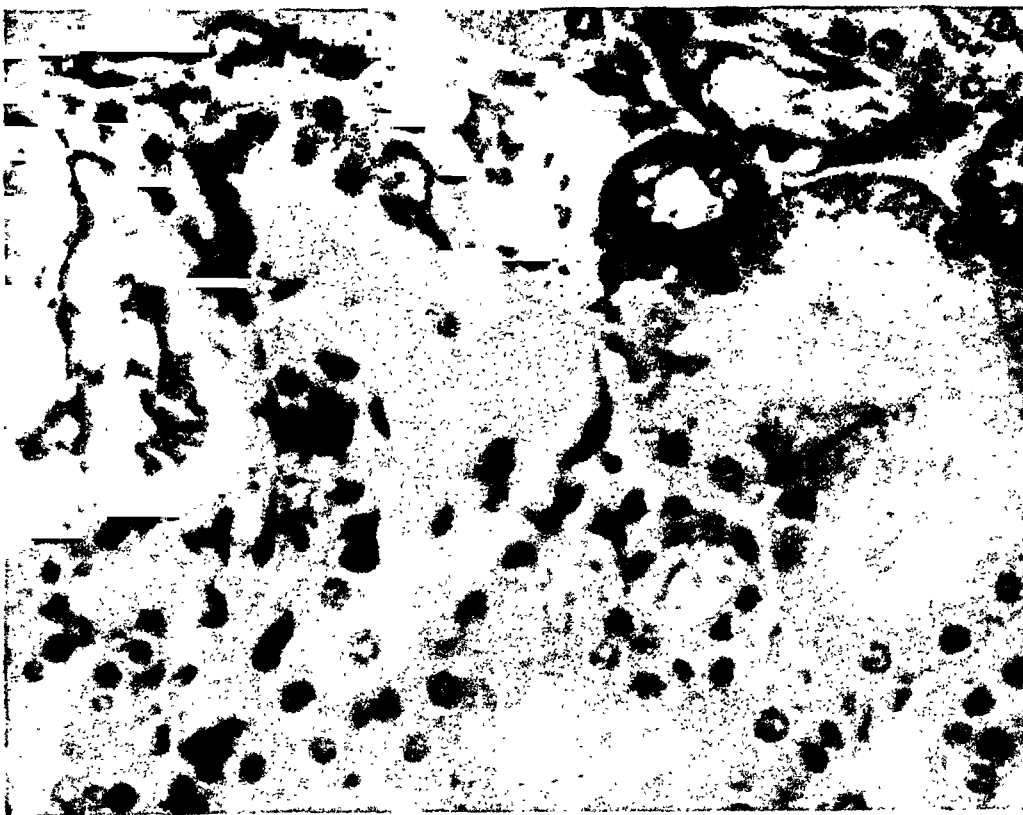
A final point worthy of attention concerns the blood vessels. In general, the cortical vessels are relatively collapsed, those of the medulla and particularly of the cortico-medullary junction dilated. From the third day on, inflammatory changes are found in the walls of the veins. As in the intertubular stroma, these are of two types: poorly circumscribed infiltrations of lymphocytes which are occasionally accompanied by polymorphonuclears and eosinophils, and sharply circumscribed granulomata of histiocytes, with or without a hyaline center. On occasion, the hyalin is extruded into the lumen of the vein where it forms the basis of a thrombus (Fig. 12). In other cases, ordinary fibrin and platelet thrombi

FIG. 5. Exudation of leukocytes and focal dilation and rupture of an ascending limb with newly regenerated atypical epithelium. Eleven-day lesion.

FIG. 6. A nonpigmented hyaline cast in an ascending limb about to be extruded from the tubular lumen. Six days.



5



6

develop above an inflammatory focus of the first type. They rarely grow to sufficient size completely to occlude the vessel.

DISCUSSION

Four theories have been advanced to explain the pathogenesis of hemoglobinuric nephrosis: mechanical blockage by precipitated hemoglobin, hyperergic inflammation, "toxic" injury and renal ischemia. Let us consider them successively.

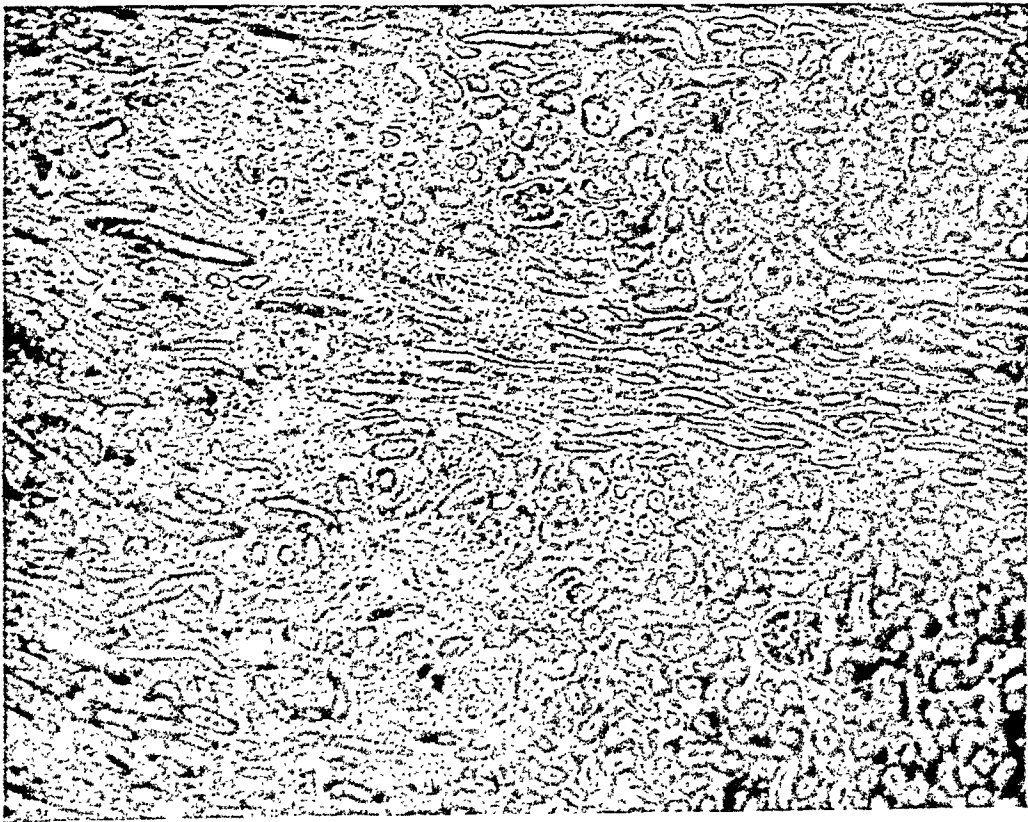
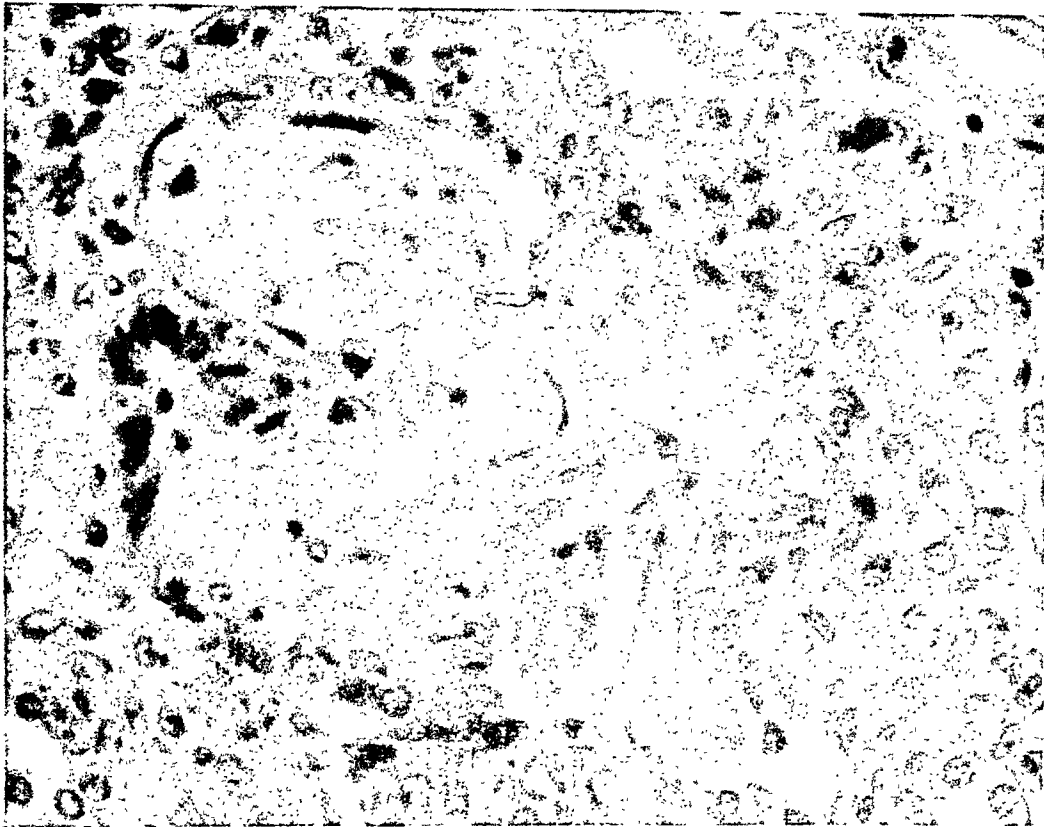
(1) The first theory is that of mechanical blockage of the tubules by precipitated hemoglobin or closely related substances. In 1925, Baker and Dodds¹ showed that hemoglobin injected intravenously in dogs was excreted by the kidneys and precipitated in the renal tubules when the reaction of the urine was acid. In such cases fatal renal insufficiency developed, whereas, if the urine were kept alkaline, the animals survived. This work was confirmed with certain reservations by DeGowin and his collaborators.^{9, 10} The simplicity of this explanation of the pathogenesis of the lesion made a wide appeal and it was generally accepted as the explanation of anuria following hemolytic crises such as transfusion reactions and blackwater fever. Recently, Bywaters and Beall,⁵ and Bywaters and Dible,⁶ have invoked the same mechanism as partial explanation of the crush kidney, the offending pigment in this instance being the closely related muscle hemoglobin or myoglobin. Grave objections have, however, been raised to this hypothesis. Van Slyke *et al.*,¹⁸ Bing,³ and Yuile *et al.*²⁰ and many others have been unable to produce renal insufficiency in dogs with single massive intravenous doses of hemoglobin regardless of the reaction of the urine. Human patients with paroxysmal hemoglobinuria, march hemoglobinuria and nocturnal hemoglobinuria do not ordinarily develop renal complications. Macgrath¹⁵ has observed fatal anuria in blackwater fever in the presence of consistently alkaline urine. The proportion of plugged nephrons is frequently inadequate to explain the almost total anuria, and conversely, kidneys in which a high proportion of the nephrons are blocked have been found at autopsy in patients who have not shown anuria.

(2) An allergic factor is suggested particularly by the cases of hemoglobinuric nephrosis which occasionally follow sulfonamide therapy. The medical ailment for which the drug is prescribed is often trivial and development of the lesion appears to be virtually independent of dosage. The possibility that anaphylaxis contributes to the reaction to "incompatible" blood has also been repeatedly suggested.¹²

(3) Recently, a new theory has been advanced which ascribes the renal damage to ischemia. Lauson¹³ and Cournand⁸ and their associates have demonstrated renal ischemia and coincident depression of renal function in patients with trau-

FIG. 7. A later stage in extrusion. The right-hand wall of the tubule has been destroyed and a granulomatous reaction is developing about the cast. Seven days.

FIG. 8. Low power of cortico-medullary junction showing the zone of maximal degenerative and inflammatory changes. There is moderate stromal edema and both diffuse and focal inflammatory reaction. Seven days.



matic and hemorrhagic shock. Phillips, Van Slyke¹⁷ and their collaborators have made similar observations in experimentally induced shock in dogs. Following prolonged shock, evidence of renal insufficiency might persist for three or four days, but they were never able to produce in their animals, by shock alone, a progressive fatal renal insufficiency such as occurs in man. In further attempts to reproduce the human lesion, another series of experiments was undertaken¹⁸ in which one kidney was removed and the renal artery on the other side was clamped for varying periods of time. When the clamp was left on for three hours, a transient renal insufficiency lasting two or three days was demonstrated. With four hours of total ischemia, half of the animals died in uremia and, with interruption of renal blood flow for six hours, none of the animals recovered. The functional results parallel those seen in human material, but the anatomic lesion is in my experience quite dissimilar. In six dogs in which the renal arteries were clamped for periods varying from two to six hours, I found diffuse degenerative changes ranging up to complete necrosis of the *proximal* tubules, but insignificant changes in the lower nephron and no formation of pigment casts.

(4) The "toxic" theory has been advanced chiefly because of dissatisfaction with the theories listed above. The authors^{16, 19} assume that toxic substances are elaborated in areas of tissue damage or of interstitial hemorrhage which injure the kidney as they are excreted through it. No direct evidence of a toxic factor has been produced. That trauma and hemorrhage are not necessary for the development of the lesion is shown by cases which have followed heat stroke and high altitude anoxemia.

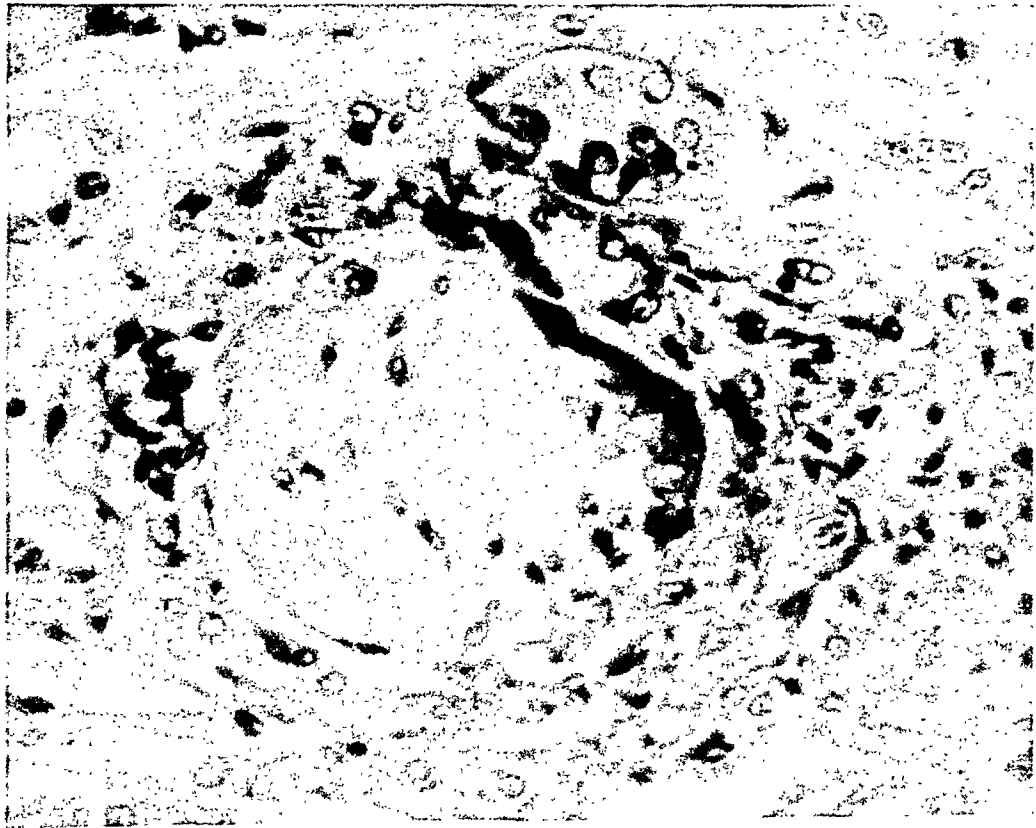
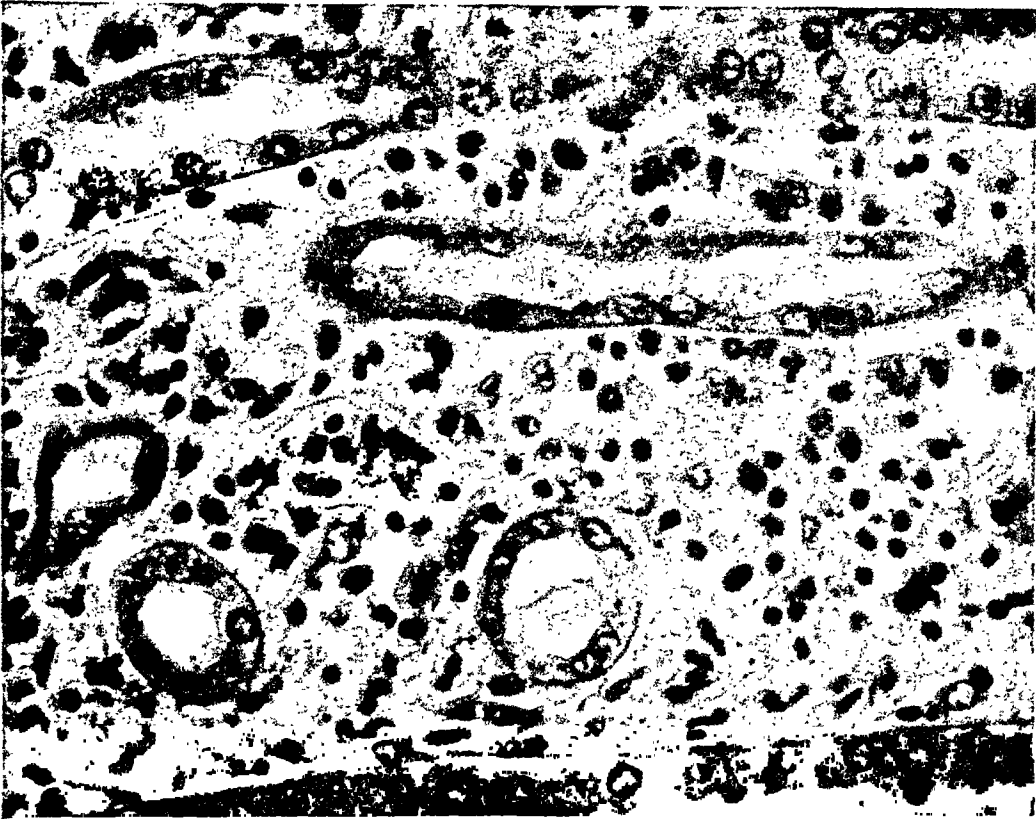
Since no single factor appears to explain adequately the development of a hemoglobinuric nephrosis, the possibility of synergistic factors deserves exploration. Two groups of investigators have approached it from the experimental side. Yuile, Gold and Hinds²⁰ produced minor grades of tubular injury by clamping the renal artery for a period of fifteen to twenty-five minutes and also by the use of potassium tartrate. A solution of hemoglobin was then instilled intravenously. When the right degree of tubular damage, neither too little nor too great, was achieved, extensive precipitation of hemoglobin and progressive renal insufficiency developed. The lesion was more severe when the urine was kept acid than when it became alkaline. Corcoran and Page⁷ achieved the same results in another manner. Shock was produced in rats by the tourniquet method; then metamyoglobin was given intravenously. Neither factor alone produced the lesions, but the combination was effective.

Our own clinical observations of the development of hemoglobinuric nephrosis in battle casualties indicate that two factors are of outstanding importance, pigment excretion and recovery from a state of shock. Let us first examine the factor of shock.

In surveying autopsies submitted from various types of Army hospitals, it was

Fig. 9. Detail of interstitial inflammatory reaction showing predominance of lymphocytes and plasma cells. Seven days.

Fig. 10. A recently extruded cast surrounded by a granulomatous reaction with a giant cell on the right. A few phagocytes have invaded the substance of the cast. Eight days.



found that hemoglobinuric nephrosis was present in only 6 per cent of cases submitted from base hospitals, 18 per cent from evacuation hospitals, and 30 per cent from field hospitals (to which only the most severely wounded, nontransportable cases were admitted). Clearly pigment nephropathy occurs in direct relation to severity of injury and by implication to profundity of shock. Further evidence was provided by our own clinical studies. One hundred and sixty-five casualties were personally examined by my clinical associates who made clinical estimates of the severity of shock. The results are shown in Table 1. The decreasing frequency of renal involvement as one passes from severe to slight grades of shock is apparent, but the 5 cases which were not considered to be in shock confuse the picture. Let us examine this group in detail. Case 9 of the series was an example of a frank transfusion accident; 125 cc. of A blood was given in error to an O recipient before a characteristic reaction developed, and the transfusion was interrupted. The possibility of anaphylactoid shock must be considered. Cases 69, 70 and 132 were examples of the crush syndrome with severe grades of myoglobinuria. Judged by the usual clinical criteria, blood

TABLE 1
CORRELATION OF GRADE OF SHOCK WITH NEPHROPATHY

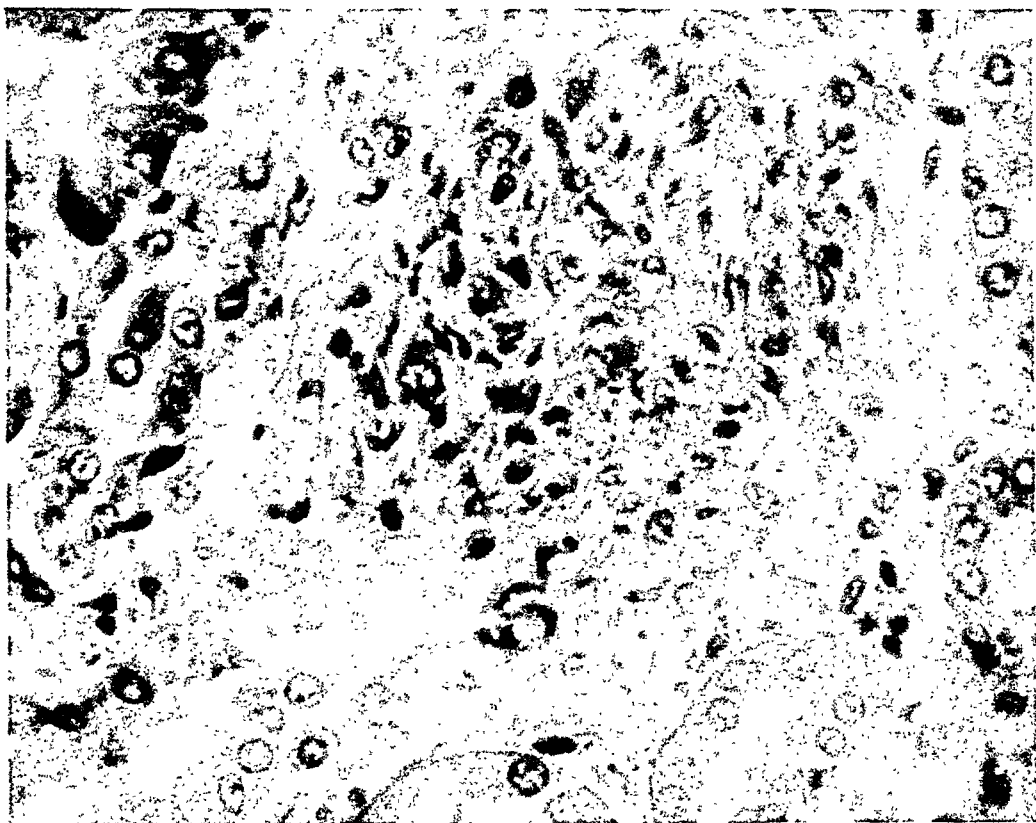
GRADE OF SHOCK	NUMBER OF CASES	FATAL NEPHROPATHIES	PER CENT
Severe.....	42	14	33.3
Moderate.....	65	15	23.1
Slight.....	36	4	11.1
None.....	26	5	19.2

pressure, pulse, pallor, coldness of the extremities, skin circulation, these patients did not impress the examiner as being in shock. Each of them, however, exhibited one phenomenon, hemoconcentration, as shown by hematocrit readings of 57, 71 and 80 respectively, which is emphasized by many students of shock. If shock itself was not present, no doubt a closely allied physiologic abnormality was.

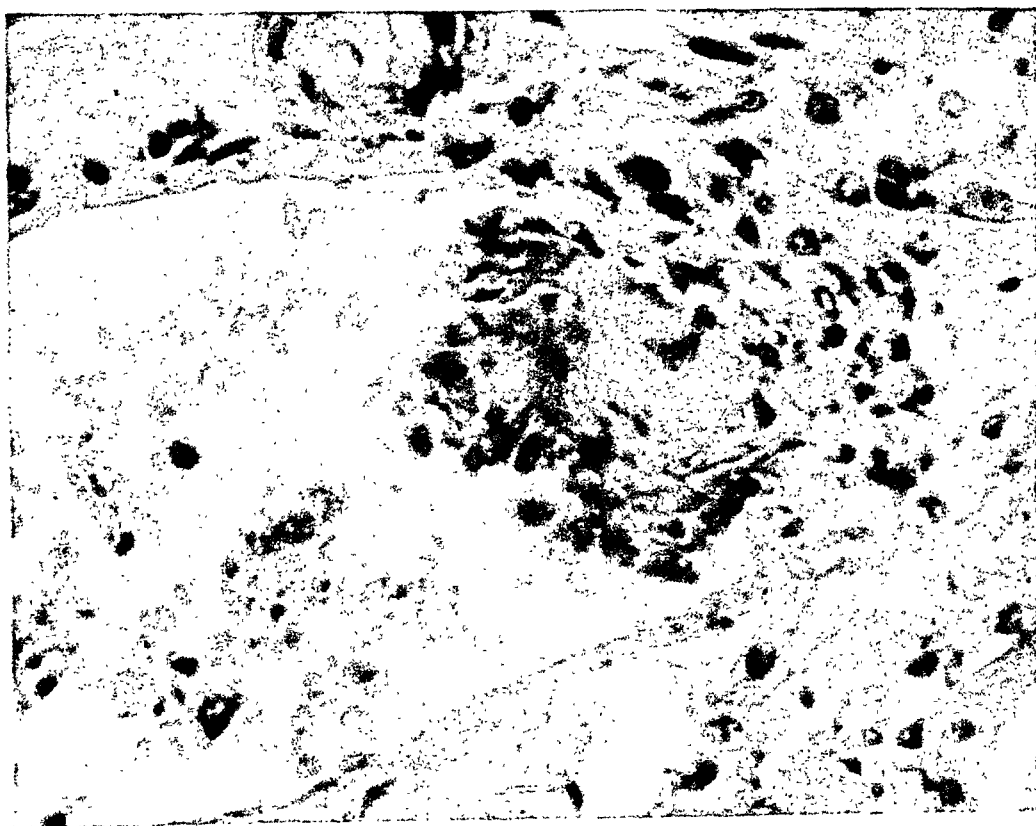
In the fifth case the soldier was wounded in the right thigh by a shell fragment which completely transected the femoral artery, vein and nerve. Hemorrhage was severe and he was given 1000 cc. of plasma en route to the hospital. On arrival, he seemed in good condition and was operated on without further preparation, but 1500 cc. of whole blood was given during the operation. Immediately after operation the blood pressure was 110/62, but seven hours later it dropped to 70/40 and throughout the day the systolic level ranged from 60 to 70. Nevertheless, the patient's color was good and his skin remained warm. The pathologist has the temerity to suggest that shock can scarcely be excluded in this case

FIG. 11. Late stage of granulomatous reaction. Only fragmentary remnants of the cast persist between the phagocytes. Ten days.

FIG. 12. Granulomatous reaction in the wall of a vein with an overlying mural thrombus. Ten days.



11



12

also. It seems fair to conclude that all our patients had suffered from shock or some closely related physiologic state.

As has already been stated, evidence of pigment excretion was found in all of our patients from whom an early specimen of urine was available. What is the source of the pigment? There are three possibilities: myoglobin from degenerating muscles, hemoglobin from lysis of the recipient's own red blood cells, or hemoglobin from lysis of red cells supplied in transfused blood.

We did not have available a spectroscope of sufficient sensitivity to distinguish the two closely related pigments. There is, however, a chemical method based upon the greater stability of myoglobin in alkaline solutions which permits a rough quantitative separation (about 80 per cent accuracy) of the two substances. This was applied in 51 wounded patients in whom measurable amounts of benzidine-positive material were present in the urine. Myoglobin was identified in 14 cases and was the dominant pigment in 11. In 7 other cases, the proportion of apparent myoglobin was so small that the results were classified as doubtful and 31 were considered negative. Except in the crush cases, we were unable to predict from the nature of the injury what type of pigment would be found in the urine. Neither massive trauma to the extremities nor interruption of a major vessel usually resulted in predominant myoglobinuria.

Since myoglobinuria can apparently explain only a small proportion of cases, causes of hemoglobinuria must be considered. With the single exception of Case 9, the transfusion accident, no instances of massive hemolysis occurred in our series. Plasma hemoglobins were usually slightly to moderately elevated even before transfusion and no marked increases occurred following transfusion. All patients except Case 9 received type O blood preserved in glucose-citrate, refrigerated, and not more than eight days old. Many flasks were directly checked for hemolysis at the moment of administration. If agglutinins for A cells were present in a dilution higher than 1 to 80, the blood was used for O recipients only. The Rh factor was not determined, but none of the patients had had previous transfusions. Cases of nephropathy developed in patients of all blood groups in a frequency proportionate to their distribution in the general population. Hemolysis due to isoagglutinins would seem to be excluded.

The majority of our patients were severely traumatized; hemorrhage into injured tissues was a usual phenomenon and could serve as a mechanism of hemolysis. Hasty conclusions that such a mechanism was of importance would be unjustified. Very few of our patients developed jaundice and it is worth re-emphasis that characteristic hemoglobinuric nephrosis develops in nontraumatic shock following such factors as heat stroke and anoxia, and incidentally without the administration of whole blood or even plasma.

CLINICOPATHOLOGIC CORRELATION

Any attempt to correlate form and function in the kidney is hazardous in the extreme but its fascination is irresistible. The first effect of shock upon the function of the kidney is oliguria followed quickly by retention of metabolic products. Both of these effects are manifested within a few hours after onset, whereas the

first recognizable morphologic change, the appearance of lipid vacuoles in the ascending loops, develops about eighteen hours after injury and pigment casts appear still later. The initial renal insufficiency is, therefore, functional rather than structural in basis. The studies of Cournand and his associates⁵ have established the early onset of renal ischemia in shock and adequately explained the initial functional derangement.

Ischemia with consequent anoxia also serves as a logical explanation of the appearance of demonstrable lipid in the parenchymal cells since a similar phenomenon is found in the liver and heart in the same cases. Whether or not the renal ischemia is sufficiently persistent to account for the later development of tubular necrosis is still unknown.

An interesting feature is the apparent delay in the appearance of the pigment casts. They were present in significant numbers in only 2 of the 11 cases in our "Board" series dying of injury within seventy-two hours. It is obvious from examination of the histories of this group that not more than one of them was ever adequately resuscitated from shock. A simple explanation for this phenomenon would be the suppression of glomerular filtration during shock either because of depressed systemic blood pressure or because of specific renal ischemia. The presence of pigment casts may therefore be considered histologic evidence of at least temporary resumption of glomerular function.

Although it is certain that the pigment casts play no rôle in the initiation of renal insufficiency following shock, no such dogmatism is possible in defining their possible effect in the later stages of the lesion. Controversy hinges on the thesis that the casts cause suppression of urine primarily by mechanical blockage of the tubules. The evidence for this is purely morphologic and at best inconclusive. It is suggested by the high proportion of apparently blocked tubules in certain cases and by the dilation of the proximal tubule. Its importance is rendered dubious by other cases in which the proportion of occluded nephrons is certainly inadequate to explain the almost total anuria.

The other histologic factors which might explain the progressive renal insufficiency are the interstitial inflammation and the tubular degeneration. It is difficult to understand how interstitial inflammation would be effective except by the production of edema, but edema is inconstant and rarely severe. Severe tubular degeneration, at least of the ascending limbs, is constant from the fourth day onward. It has been suggested that injured tubular cells permit rediffusion of glomerular filtrate back into the vascular system. With such a mechanism one might, however, expect as in lipoid nephrosis a high-gravity oliguria rather than the low, fixed gravity which is characteristic of these cases. The focal rupture of tubules, so frequently noted from the fifth day onward has to my mind little functional significance. It is a late phenomenon, whereas the entire syndrome, oliguria, azotemia, pigment excretion, hypertension and fixation of gravity is established before it appears.

SUMMARY

The clinical and pathologic features of the syndrome of renal insufficiency which develops in patients resuscitated from shock have been described. The

constant clinical features are oliguria, azotemia, excretion of pigment and albumin in an acid urine, fixation of specific gravity. Inconstant but usual, and diagnostically of great importance, is the appearance of mild hypertension on the third or fourth day.

The histologic features develop in orderly sequence. The initial change appearing eighteen to twenty-four hours after injury is lipid vacuolization of the ascending limbs of Henle's loop. The second stage is precipitation of pigment, either hemoglobin or myoglobin, in the distal convoluted and collecting tubules. This rarely occurs short of twenty-four hours, more commonly between thirty-six hours and seventy-two hours after injury. Moderate dilation of proximal and sometimes distal convoluted tubules follows pigment precipitation. Sometimes on the third day, regularly on the fourth and fifth days, necrosis and regeneration of epithelium in the ascending limbs and distal tubules become evident and simultaneously lymphocytes appear between the tubules and about the vessels. From the fifth day on, rupture of tubules with herniation of their content into the stroma and consequent granuloma formation becomes frequent. The last development in many cases is the formation of nonocclusive mural thrombi in many of the small veins.

In clinicopathologic correlation it was shown that two factors were constant: the excretion of benzidine-positive pigment in the urine and recovery from a state of shock or related physiologic abnormality. Renal insufficiency was found to antedate all structural change but was never progressive in the absence of a demonstrable pigment nephropathy. Although pigment precipitation is an essential factor in the development of the "shock kidney", the mechanism of its effect remains unelucidated. The possibility that it is in part mechanical cannot be discarded, but the complex chain of degenerative and inflammatory phenomena demand another explanation. Further controlled experimentation will be necessary before many of these uncertainties can be resolved.

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STUDIES ON PHAGOCYTOSIS*

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It is accepted by many histologists that the property of engulfing foreign substances is not a monopoly of phagocytes and that phagocytes differ from other cells only in their greater capacity for engulfment and in the rapidity with which they work. Metchnikoff³ ascribed phagocytic properties to muscle cells (myocytes) which, in the tadpole, devour the sarcoplasm of the skeletal muscle during the involution of the tail. Later, Forbus⁴ observed that myocytes phagocytosed intravitaly introduced carmine. Phagocytosis by myoblasts has also been studied in tissue cultures by Levi and Buccianti.⁷ On the other hand, Pfuhl⁹ denied a phagocytic function of myocytes (dissociated muscle cells). Recently, Altschul¹ found that in experimental arteriosclerosis, lipoids appear in muscle cells which turn into "foam cells", but it remains to be decided whether this process is to be regarded as phagocytosis by the muscle cells, or as lipoid degeneration.

A simple way to study alterations of skeletal muscle experimentally is to insert catgut threads into the muscle for various periods of time.² Some of the fibers become necrotic, hyalinized and calcified. Others, less affected, show a nuclear proliferation (Fig. 1A) and eventually a dissociation into more or less individualized muscle cells. If prior to insertion, the catgut thread has been impregnated with any substance, the effect of this substance on the injured muscle fibers may be determined. Some observations of this character are reported here.

MATERIAL AND METHODS

The animals used were rabbits, guinea pigs, white rats, chickens and frogs. After impregnation with trypan blue (1 per cent and 10 per cent), carmine (5 per cent), hematoxylin (10 per cent), India ink, neutral red, Janus green or gentian violet, the catgut threads were dried and inserted into the muscle. Of the substances just mentioned, only the first four were phagocytosed. The results with the last three were negative and have been omitted in this discussion.

In five animals, the procedure was modified as follows: (a) In one white rat, hematoxylin-impregnated catgut was inserted into the liver and left there for twenty days. (b) In two white rats, hematoxylin-impregnated threads were inserted into the brain and the animals killed after ten and twenty days, respectively. (c) In one rabbit, trypan blue injections were given intraperitoneally ten weeks after avulsion of one sciatic nerve. (d) In another rabbit, trypan blue injections were given intravenously ten days after a sterile abscess had been set up in the muscle of one hind limb.

RESULTS AND DISCUSSION

Dissociating muscle cells of damaged, but not completely necrotic, muscle fibers, took up the dye following the insertion of catgut impregnated with either

* Supported by a grant from the Associate Committee on Army Medical Research, National Research Council, Ottawa. Received for publication, March 26, 1947.



FIG. 1A. "Giant muscle cell" from neighborhood of a catgut thread soaked with hematoxylin. Note hematoxylin granules around the proliferated muscle nuclei. White rat. Hematoxylin-eosin stain.

FIG. 1B. "Giant muscle cell" from the periphery of a sterile abscess. Intravenously injected trypan blue was taken up around the proliferated muscle nuclei. The center of the formation consists of hyalinized sarco-plasm and fibrillae. Note scattered histiocytes. Rabbit. Counterstained with eosin.

trypan blue, carmine, hematoxylin (Fig. 1A), or India ink and in rabbit (d) with the sterile abscess, following the intravenous injection of trypan blue (Fig. 1B). So also, as was to be expected, did numerous histiocytes of the interstitial tissue. The quantity engulfed was much greater with trypan blue and hematoxylin than with carmine or India ink. Hyalinized muscle fibers were diffusely stained by trypan blue and were stained to an even greater extent by hematoxylin, but such staining was not observed with carmine or India ink.

In the rabbit which received trypan blue intraperitoneally, the proliferated muscle nuclei did not show granules of trypan blue in the juxtannuclear plasma. It may be assumed either that the intact sarcolemma prevented the penetration of the dye or that the myocytes were not sufficiently individualized. In the aforementioned experiments, a similar lack of phagocytosis by muscle cells in apparently undamaged fibers was observed with local application of the dyes.

A point of special interest was the loss of some of the histochemical properties of hematoxylin, which had been introduced intravitaly. The hematoxylin was found as rusty brown granules in the histiocytes of the interstitial spaces, in myocytes and diffusely in necrotic muscle fibers. Contrary to our expectation, we were unable to "blue" these hematoxylin granules by treating the sections with tap water, lithium carbonate water, or ammonia vapors. Neither were we able to "differentiate out" the hematoxylin by treating the sections up to forty-eight hours with 0.1 per cent hydrochloric acid or with a saturated aqueous solution of picric acid. Treatment with 4 per cent violet ammonium alum or iron chloride failed to darken the granules by forming a lake of the iron salt and the hematoxylin, or to "differentiate out" their color. Weigert's neuroglia mordant darkened the granules slightly. The only way that we found to remove the color from the sections was by prolonged treatment with 0.25 per cent potassium permanganate, followed by bleaching in a mixture of equal parts of 1 per cent potassium sulfite and 1 per cent oxalic acid. If applied without previous potassium permanganate treatment, the potassium sulfite-oxalic acid mixture had no effect.

In the liver and brains of the rats in which hematoxylin threads had been inserted, the dye was taken up by the various types of histiocytes and showed the same loss of histochemical properties as just described for the experiments on skeletal muscle.

It appeared possible that the firm retention of the color was due to the secondary action of the fixative. If this were so, postvitaly stained tissue should show the same reactions as intravitaly stained tissue. It was proved by control experiments that this was not the case, but that postvitaly stained tissue shows staining properties similar to sections which have been fixed, embedded, cut and stained in the usual manner. In these controls, small pieces of fresh tissue were stained twenty-four hours and ten days respectively in a 10 per cent aqueous solution of water-soluble hematoxylin, then fixed in formalin and embedded in paraffin. The sections showed the same rusty color seen in the intravital preparations, but were readily "blued" in tap water or with ammonia vapors. Iron ammonium alum and iron chloride blackened them first, but prolonged

treatment "differentiated out" the color. The sections were darkened by Weigert's neuroglia mordant and by potassium bichromate. Saturated picric acid or 0.1 per cent hydrochloric acid removed some of the color, and the sections were subsequently blued with tap water. Potassium permanganate, followed by the potassium sulfite-oxalic acid mixture completely removed the hematoxylin.

From these experiments, it appears obvious that the hematoxylin stain adheres very firmly to tissues, if applied intravitaly, and also that it does not change if acid, alkaline solutions, iron, or chrome salts act upon it. All this is in contrast to the hematoxylin staining of dead tissue, whether used in routine staining or in a postvital method, and when "bluing" by alkalinizing, differentiation by acids, and lake formation with mordants are readily attained. In explanation of these differences, we are inclined to believe that intravitaly absorbed hematoxylin becomes intimately combined with proteins of tissue fluids and of living and dead structures, thereby losing certain of its histochemical reactions. This intimate union is in contrast to the looser combination which results if the stain is applied after death.

Observations regarding the fate of intravitaly introduced dyes, starch and colloidal metals were previously reported by Bennhold,² although it appears that these substances were studied only after introduction directly into the blood stream. More recently, Knisely, Bloch and Warner⁵ observed the plasma coating of India ink, kaolin, graphite particles and mercuric sulfide, which had been injected into the vascular system of frogs. They came to the conclusion that without a plasma coating no phagocytosis of these substances occurs, at least not by the hepatic phagocytes. In addition to the aforementioned substances, red blood cells containing parasites in Knowlesi malaria also received a coating and were then singled out by phagocytes (Knisely, Stratman-Thomas, Eliot and Bloch⁶).

SUMMARY

The phagocytic action of muscle cells in damaged fibers of skeletal muscle is confirmed. If catgut threads impregnated with hematoxylin are inserted into skeletal muscle, liver or brain, the dye is taken up by phagocytes, myocytes and necrotic tissue, and loses most of its characteristic histochemical reactions. It is suggested that this may be due to a coating by protein, which protects the hematoxylin from the chemicals with which it ordinarily reacts in routine staining. Such a coating has been repeatedly reported for other substances.

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AGNOGENIC MYELOID METAPLASIA OF THE SPLEEN

REPORT OF CASE WITH NECROPSY FINDINGS*

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Jackson, Parker and Lemon² described a syndrome of extramedullary myeloid metaplasia which they designated as agnogenic.† Because this condition is often confused with myelogenous leukemia or acquired hemolytic jaundice, these authors separated agnogenic myeloid metaplasia of the spleen from other conditions which it may simulate, and further showed that patients died soon after irradiation was administered or splenectomy was performed.

Extramedullary myelopoiesis may occur in a great variety of pathologic conditions, and has been observed in various infectious and septic states such as metastatic carcinoma to bone and leukemia. The distinctive features in these types of extramedullary myelopoiesis are that the apparent clinical and pathologic disorders are known and the therapy and prognosis are obvious.

In agnogenic myeloid metaplasia the diagnosis may not always be established, but often one can, by hematologic methods (peripheral, sternal and splenic puncture), clinical examinations and, later, by histologic studies of the spleen, separate this entity from others simulating it.

The essential clinical findings are as follows: progressive weakness associated with abdominal distress, enlargement of the spleen, tendency toward hemorrhages and a slightly elevated or lowered white count with the presence of immature red and white cells in the peripheral blood.

Jackson and his associates reported 10 cases which they had under observation. In three, a diagnosis of myeloid metaplasia was made and the treatment was symptomatic. These patients are alive. In one patient, a diagnosis of hemolytic jaundice was made, splenectomy was performed and the patient expired three days postoperatively. In two instances, no diagnosis was made, splenectomy was performed and the patients died within a year. In four patients, a definite diagnosis of myelogenous leukemia was made; one was treated symptomatically and expired three years later; three were treated by roentgen rays. Two of the latter patients died within six weeks and the third, who derived no benefit from the therapy, died from bronchopneumonia two years following therapy.

Reich and Rumsey³ reported 5 cases of agnogenic myeloid metaplasia of the spleen, illustrating diagnostic difficulties and the danger of splenectomy and radiation therapy. In not one of these cases was the clinical and pathologic diagnosis established. The clinical diagnoses were splenic anemia in four in-

* Received for publication, March 4, 1947.

† The term agnogenic (derived from the Greek "of unknown or uncertain etiology") was suggested by Dr. Robert M. Green.

stances and leukemia in one. The pathologic diagnosis of the excised spleen was either Hodgkin's disease or atypical leukemia. These authors, therefore, came to the conclusions that the diagnosis of agnogenic myeloid metaplasia of the spleen is difficult, that the condition must be differentiated from Banti's disease, hemolytic anemia and leukemia, and that splenectomy and irradiation therapy are definitely contraindicated.

The present case was diagnosed clinically as agnogenic myeloid metaplasia of the spleen after studies of the bone marrow and peripheral blood. This diagnosis was corroborated by the histologic examination of the spleen and later by necropsy findings. This case is also of interest because of the development of hemosiderosis which is explained by the multiple (40) blood transfusions that the patient received.

REPORT OF CASE

Clinical data. A 49 year old Polish housewife upon admission to the hospital complained of weakness, dyspnea on exertion, intermittent pains over the heart and lower portion of the chest, and loss of 60 pounds in weight. When these symptoms appeared three years earlier, she visited her family physician, who told her that she was anemic and gave her a series of injections for a period of four months. She later was taken to a hospital where she was given blood transfusions. She was a patient in three different hospitals in Chicago, where splenic puncture and bone marrow studies were made. Over a period of two years prior to her admittance to the Research and Educational Hospitals, she received 40 transfusions. During this time, the following diagnoses were made: aplastic anemia, Hodgkin's disease, leukemia, carcinoma of the pancreas and Banti's syndrome. Her past history was essentially negative.

Her skin was dark in color but the conjunctiva and mucous membranes were pale. Blood pressure was 112/64, temperature 98.6 F., pulse rate 84 and respiratory rate 20. Except for a systolic murmur at the apex, the heart and lung findings were negative. The spleen was palpated 5 fingerbreadths below the costal margin. The liver was tender and palpated 3 fingerbreadths above the right iliac crest. The remainder of the examination of the abdominal organs and extremities was negative.

The peripheral blood and bone marrow studies (Fig. 1) made on the day of admission are recorded in Tables 1 and 2. It will be noted that the peripheral blood pattern was that of a leukemoid reaction and the bone marrow was hypercellular (myeloid-erythroid volume)⁵ and revealed a marked erythroid immaturity. There was a moderate myeloid immaturity of the marrow, but this did not extend to the stage of myeloblastic involvement. The megakaryocytes were increased in number but presented a normal maturation dispersion. The reticulum cells and macrophages were also increased in number.

There was difficulty in cross matching the patient's blood. Following her first transfusion of 500 cc. of blood after entering the Research and Educational Hospitals, she complained of much tearing of the eyes and swelling of the lids. Fifteen days following her admission, the abdomen became distended and there was shifting dullness. Ankle edema and sacral edema were present. At this time, she received her fourth pint of blood. The patient was type O, Rh-positive, and attempts to cross match her blood with that of various donors had been unsuccessful, with a few exceptions. On one or two occasions, when the patient received blood transfusions, her temperature became elevated to 102 F., but there were no chills or malaise.

The clinical diagnosis was agnogenic myeloid metaplasia of the spleen.

About six weeks after admission a splenectomy was performed. The spleen weighed 520 gm. and measured 20 by 9.5 by 6 cm. The capsule was smooth, glistening and mottled red. The cut surfaces showed a cherry red coloration of the stroma.

Microscopic findings. The sections revealed some capsular and trabecular fibrous thickening. There were fairly large lymphoid follicles but these were widely separated. Some of them revealed irregular central fibrosis and many histiocytes. The pulp was characterized by wide sinusoids lined by prominent endothelium. Some of these sinusoids were filled with young hemopoietic cells. The pulp surfaces between the Malpighian corpuscles were wide and the degree of cellularity varied from field to field. In addition to the usual type of cells, however, there were collections of larger cells and histiocytes laden with hemosiderin. Most of the young cell forms revealed little evidence of maturation. There was

TABLE 1
PERIPHERAL BLOOD STUDIES

	BEFORE SPLENECTOMY (JAN. 28, 1946)	AFTER SPLENECTOMY (MARCH 31, 1946)	PRIOR TO DEATH (APRIL 19, 1946)
Hemoglobin (gm.)	2.1	7.4	1.5
Erythrocytes (millions)	0.89	3.15	0.64
Leukocytes	13,950	33,500	14,500
Hematocrit (erythrocyte per cent)	7.0	28.0	5.0
Hematocrit (buffy per cent)	1.5	3.0	1.5
Sedimentation rate (mm./hr.)	88.0	55.0	86.0
Corrected rate (mm./hr.)	8.0	28.0	2.0
Mean corpuscular volume (C.M.)	78.6	88.0	78.0
Mean corpuscular hemoglobin ($\mu\mu$)	23.5	23.0	23.0
Mean corpuscular hemoglobin concentration (per cent)	30.0	30.0	30.0
Reticulocytes (per cent)	0.5	0.8	0.5
Icterus index (units)	10.0	20.0	20.0
Platelets	Increased	Normal	Increased
Differential Smear			
Myeloblasts	3.0	0.0	0.0
Promyelocytes	2.0	0.0	0.0
Neutrophilic myelocytes	21.0	15.0	8.0
Neutrophilic metamyelocytes	33.0	16.0	4.0
Stab neutrophils	24.0	9.0	4.0
Polymorphonuclear neutrophils	4.0	49.0	70.0
Eosinophils	0.0	1.0	0.0
Basophils	0.0	0.0	0.0
Lymphocytes	10.0	1.0	2.0
Monocytes	3.0	9.0	12.0
Normoblasts (per 100 leukocytes)	3.0	4.0	1.0

an occasional multinucleated cell which had megakaryocytic qualities (Fig. 2). A portion of the tail of the pancreas showed abundant interstitial hemosiderin with pigment deposits in the parenchymal and islet cells. There were irregular, atrophic, parenchymal changes. The section taken through the splenic artery revealed relatively mild sclerotic changes with calcification.

The diagnoses were splenomegaly with myeloid metaplasia and interstitial fibrosis of the pancreas and hemosiderosis.

Twenty-four hours following operation, she developed dyspnea, fever, tachycardia and marked lag in expansion of the left side of the chest. Relief was obtained by use of a nasotracheal tube. Ten days following operation, she complained of pericardial pain. Occa-

sional extrasystoles and râles in the left lower lobe were heard. The abdomen was distended and there was edema of the arms and legs. The blood pressure was 110/70 and a pulsus paradoxicus was present. The liver was felt some 4 fingerbreadths below the costal border. Approximately 250 cc. of bloody fluid was removed by pericardial paracentesis. The red blood cell count of this fluid was 1.05 millions per cu. mm. Twelve days following surgery, there was brown pigmentation of the skin. The blood sugar content was elevated. A pleuropericardial friction rub was present and the pulse was obliterated on inspiration. Two thoracenteses were performed on the same day; the first tap yielded 1000 cc. and the second tap, 1200 cc. of bloody fluid.

TABLE 2
BONE MARROW STUDIES

	BEFORE SPLENECTOMY (JAN. 28, 1946)	AFTER SPLENECTOMY (MARCH 31, 1946)	PRIOR TO DEATH (APRIL 19, 1946)
Quantitative Values (per cent)			
Fat (yellow and red)	0.0	0.0	0.0
Plasma	80.0	53.0	72.0
Myeloid-erythroid cells*	15.0	25.0	20.0
Erythrocytes	5.0	22.0	8.0
Qualitative Findings (per cent)			
Myeloid series			
Myeloblasts	0.0	1.0	1.0
Promyelocytes†	20.0	22.0	20.0
Neutrophilic myelocytes	24.0	18.0	20.0
Neutrophilic metamyelocytes	50.0	39.0	35.0
Polymorphonuclear neutrophils	6.0	18.0	23.0
Eosinophils	0.0	2.0	1.0
Basophils	0.0	0.0	0.0
Erythroid series			
Pronormoblasts	22.0	20.0	18.0
Basophilic normoblasts	56.0	69.0	67.0
Polychromatic normoblasts	22.0	11.0	15.0
Orthochromatic normoblasts	0.0	0.0	0.0
Myeloid-erythroid ratio	1:1	3:1	3:2
Myeloid-erythroid per cent	50:50	75:25	60:40
Megakaryocytes	Increased	Increased	Increased

* Normal myeloid-erythroid volume, 6.8 per cent.

† Includes leukoblasts.

The basal metabolic rate on admission was +30 per cent; two months later, +11 per cent. X-ray films on various occasions showed diffuse cardiac enlargement, fluid in the left pleural cavity and pulmonary congestion. The urinalysis was negative. The red blood cell count varied between 1.3 and 3.5 millions per cu. mm., the white blood cell count between 8.4 and 14.1 thousands and the sedimentation rate by the Wintrobe method (corrected) varied between 8 and 10 mm. in one hour. The Kahn and Wassermann tests were negative. Cultures of the pericardial and pleural fluids were negative. The chemical findings were as follows: glucose, 190 mg.; glucose tolerance test, 97, 136, 182, 200 mg.; NPN, 21 to 50 mg.; uric acid, 3.3 to 5.0 mg.; creatinine, 1.5 mg.; carbon dioxide combining power, 56 cc. per 100 cc. blood; cholesterol, 148 mg.; sodium chloride, 487 to 551 mg.; albumin/globulin ratio, 4.0/3.5 and 3.9/2.0; icterus index, 20 units; bilirubin, 1.6 mg.

Following splenectomy and up to the time of death, the peripheral blood presented an increase in the number of polymorphonuclear neutrophils and a gradual decrease in the number of immature myeloid cells, although a few neutrophilic metamyelocytes and myelocytes and normoblasts were always present (Table 1). The bone marrow (Table 2) following splenectomy revealed a shift to the right in the myeloid dispersion which was reflected in the peripheral blood by an increase in the number of polymorphonuclear neutrophils. The dispersion of the erythroid cells remained practically unchanged. The immature cells of this series were pronormoblasts. Megaloblastic erythropoiesis was at no time observed, and there was also a lack of erythroid conversion to a normoblastic type following intramuscular liver therapy. Reticulum cells and macrophages were frequently observed in the bone marrow prior to and following the removal of the spleen (Fig. 3).

Following splenectomy, her temperature rose from normal to 103 F., but returned to normal in a few days. She received four and one-half pints of blood while in this hospital. The second pint was given following splenectomy. The red blood cell count was 3.8 millions; the white blood cell count, 8000; hemoglobin, 11.0 gm.; and hematocrit, 26.5 per cent. Three days following operation, the fragility test showed values from 0.4 to 0.55 per cent saline and no spherocytosis of the red blood cells was noted. She developed râles in both lung fields and expired three months and five days after admission to this hospital.

NECROPSY FINDINGS

The necropsy findings are reported briefly as follows. There was pitting edema of the hands, left elbow, left breast and both legs. There was brownish discoloration of the skin. In the left upper quadrant of the abdomen, there was a relatively recent surgical incision, which was almost completely healed, and there was an ancient healed scar in the lower mid-portion of the abdomen.

The peritoneal cavity contained 800 cc. of clear, slightly greenish fluid and there was a hematoma in the left rectus muscle beneath the site of the surgical incision. There were adhesions between the omentum and the more recent surgical scar and between the left lobe of the liver and the anterior abdominal wall. A walled off abscess, 2 cm. in diameter, was present between the greater curvature of the stomach, the left lobe of the liver, and the left leaf of the diaphragm. The right leaf of the diaphragm was at the level of the fourth intercostal space and the left leaf at the level of the sixth rib.

The left pleural cavity contained 500 cc. of a clear yellow fluid, the right, 100 cc. of a similar fluid, and the pericardial sac, 30 cc. of a slightly cloudy yellow fluid. The thoracic and abdominal lymph nodes were enlarged, soft and brownish. The pericardial sac was adherent to the visceral pericardium and the parietal pericardium was markedly thickened, roughened and contained deposits of fibrin. The heart weighed 475 gm. Besides the irregular pericardial deposits of fibrin with organization, there was a pale myocardium and a tigered appearance of the endocardium. The right lung weighed 390 gm. and the left, 320 gm. There was marginal atelectasis in the lower lobe of the left lung. The liver weighed 1980 gm. and showed slight accentuation of the lobular markings. The pancreas weighed 70 gm., was soft and pale brown with an area of fibrosis in the tail. The adrenal glands were brown. The right kidney weighed 200 gm., the left 240 gm. They showed smooth pale yellow surfaces. The pelves, ureters, and urinary bladder were grossly unchanged. The gastrointestinal tract was unchanged except for a pale brownish coloration of the mucosa of the stomach. The thyroid gland weighed 50 gm.

The bone marrow from the lumbar vertebrae, sternum, rib and femur was watery and pale gray throughout. Postmortem blood culture showed hemolytic *Staphylococcus aureus* and a similar organism was isolated from the peritoneal cavity.

The anatomic diagnoses were: agnogenic myeloid metaplasia of the spleen with marked anemia; splenomegaly with splenectomy; hyperplasia of the bone marrow; hemosiderosis of liver, pancreas, stomach, adrenals and lymph nodes; pigmentary cirrhosis of the liver; interstitial fibrosis of the pancreas with mild diabetes; fatty degeneration of heart and kidneys; chronic abscess of the left upper quadrant of the abdomen; subacute fibrinous peri-

carditis; left pleural effusion; atelectasis of the lower lobe of the left lung; septicemia (*Staphylococcus aureus*); hematoma of the abdominal wall; fetal adenoma of the thyroid; and pneumoliths.

DISCUSSION

Agnogenic myeloid metaplasia of the spleen can be most frequently confused with other entities associated with splenomegaly. The most frequent diagnoses considered are myelogenous leukemia in its various phases and aplastic anemias. In view of the fact that there is agreement in the treatment of agnogenic myeloid metaplasia of the spleen by symptomatic therapy only, it is very important that the diagnosis of this condition be established and separated from other clinical entities which it may simulate. This can be done by clinical, hematologic and pathologic studies.

Little is known about the etiology or pathogenesis of splenomegaly. Some have attempted to classify enlargement of the spleen with systemic affections in which the spleen shows a hyperplasia of its normal structural elements, storage of abnormal substances, or development of new tissue that may be neoplastic or inflammatory in nature. The following classification of splenomegaly is taken from Jaffé:³

1. Circulatory disturbances;
2. Hyperfunction of spleen, which includes storage and excessive destruction of erythrocytes with or without jaundice, Banti's syndrome, pernicious anemia, thrombocytolysis;
3. Excessive formation of blood cells as in myelopoiesis, which includes the leukemic and aleukemic myeloses;
4. Metabolic disturbances, such as Niemann-Pick and Gaucher's disease;
5. Specific granulomas;
6. Primary tumors.

From this group, aleukemic myelosis is the condition which is to be considered most seriously. In Jaffé's discussion of splenomegaly, he stated: "Aleukemic myelosis usually offers great diagnostic difficulties, and misinterpretation, as splenic anemia or essential thrombocytopenia is very common. The differential diagnosis is important because of the very poor prognosis of the splenectomy in aleukemic myelosis. The aleukemic form passes sometimes, especially in its terminal stages, into the leukemic form. A definite diagnosis can be secured only by splenic puncture."

According to Kracke,⁴ splenectomy should be performed when evidence points to a disturbance of the orderly destruction of red blood cells, and there is no evidence of ingested drugs or contact with noxious and chemical agents that might be responsible for the blood destruction. The conditions for which splenectomy has been recommended are trauma and hemorrhage of the spleen, primary tumors

FIG. 1A. Smear of peripheral blood showing a leukoblast, a polymorphonuclear neutrophil, and a normoblast. $\times 1100$.

FIG. 1B. Smear of bone marrow (sternal puncture) showing a hypercellular marrow with marked erythroid immaturity and a moderate left shift in the myeloid cells without myeloblastic involvement. $\times 1100$.

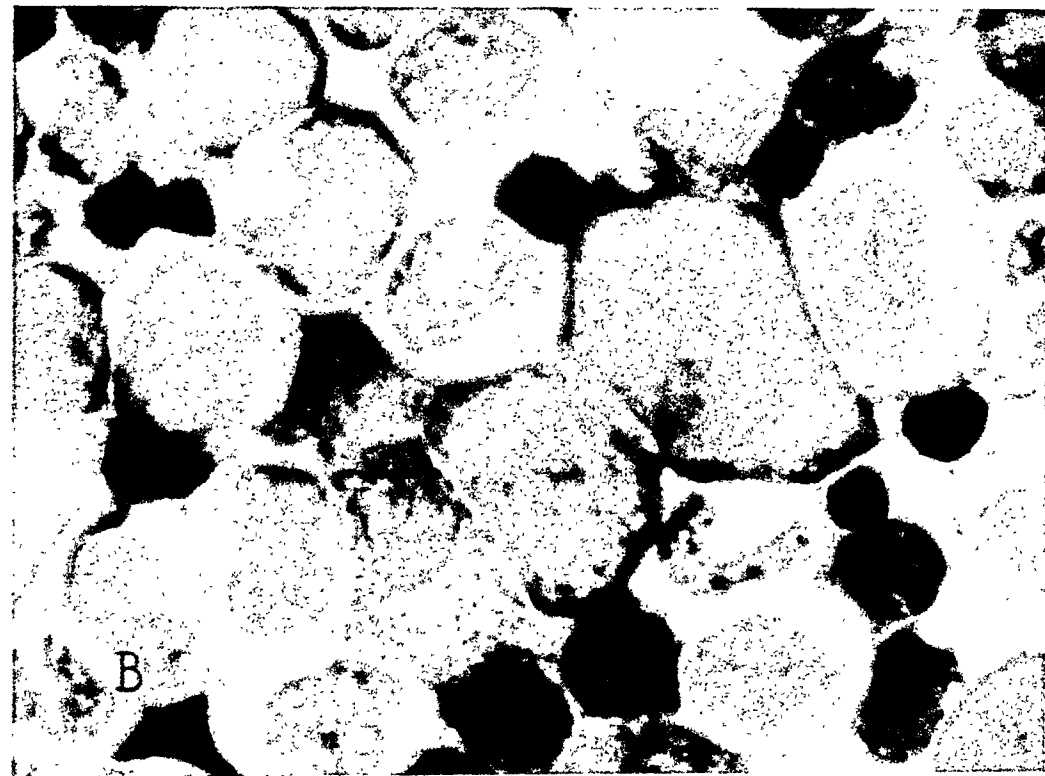


FIG. 1

of the spleen, congenital hemolytic icterus, essential thrombocytopenic purpura, Gaucher's disease, Banti's syndrome, primary splenic neutropenia and primary splenic panhematocytopenia. Agnogenic myeloid metaplasia of the spleen is not mentioned in Kracke's report.

We agree fully with the following statement from Jackson, Parker and Lemon:² "During life, the condition (agnogenic myeloid metaplasia of the spleen) may be suspected when a patient with a large spleen has had, over a considerable period of time, a subleukemic peripheral blood picture, together with occasional nucleated red cells in the smear. This suspicion becomes a probability if the spleen is very large and hard, slight jaundice is present, the course of the disease is slow and the total white-cell count is comparatively low. The disease may be properly diagnosed when, under these conditions, bone-marrow biopsy fails to reveal the pathologic changes characteristic of leukemia and splenic puncture shows the presence of megakaryocytes and immature cells of both the red-cell and white-cell series. Should the peripheral blood be suggestive and the bone marrow prove to be non-leukemic, splenic puncture is necessary in order to rule out those conditions in which splenectomy might be indicated, if only as a last resort."

Although these authors have reported 10 cases of agnogenic myeloid metaplasia of the spleen, which represents the largest number thus far reported in the literature, we may question the rationale of routine splenic puncture as a diagnostic procedure. This was performed in our patient by a physician in another hospital before the patient came to our attention and did not lend any diagnostic aid. It is possible that the technic was faulty and that, in more experienced hands, the results might have been of value. However, these two outstanding diagnostic criteria, the subleukemic to leukemic peripheral blood picture, and the absence in the biopsy of bone marrow of pathologic changes characteristic of leukemia, should be valuable aids in the diagnosis of this disease.

The bone marrow of our patient was hyperplastic. The erythroid tissue was active and showed a marked immaturity of a pronormoblastic type. The myeloid tissue revealed a moderate to marked myeloid immaturity, but did not involve the myeloblastic tissue. The megakaryocytes were increased and presented a normal dispersion, the histiocytes were frequently accompanied by phagocytic activity and no cells foreign to the bone marrow were seen.

The spleen in agnogenic myeloid metaplasia reveals capsular and trabecular fibrous thickening. The lymphoid follicles are large, widely separated, and some reveal irregular central fibrosis and histiocytes. Scattered irregularly throughout the stroma are focal areas of hematopoietic cells, myelocytes and cells of the granulocytic series. Multinucleated cells which have megakaryocytic qualities and normoblasts are present. Larger cell forms and histiocytes laden with hemosiderin are also seen. In the present case, no organ, with the exception of

Fig. 2A. Section of spleen (low power) showing Malpighian corpuscle, with islands of myeloid metaplasia. $\times 400$.

Fig. 2B. Section of spleen (oil immersion) showing a megakaryocyte surrounded by immature types of myeloid cells and a few normoblasts. $\times 1100$.

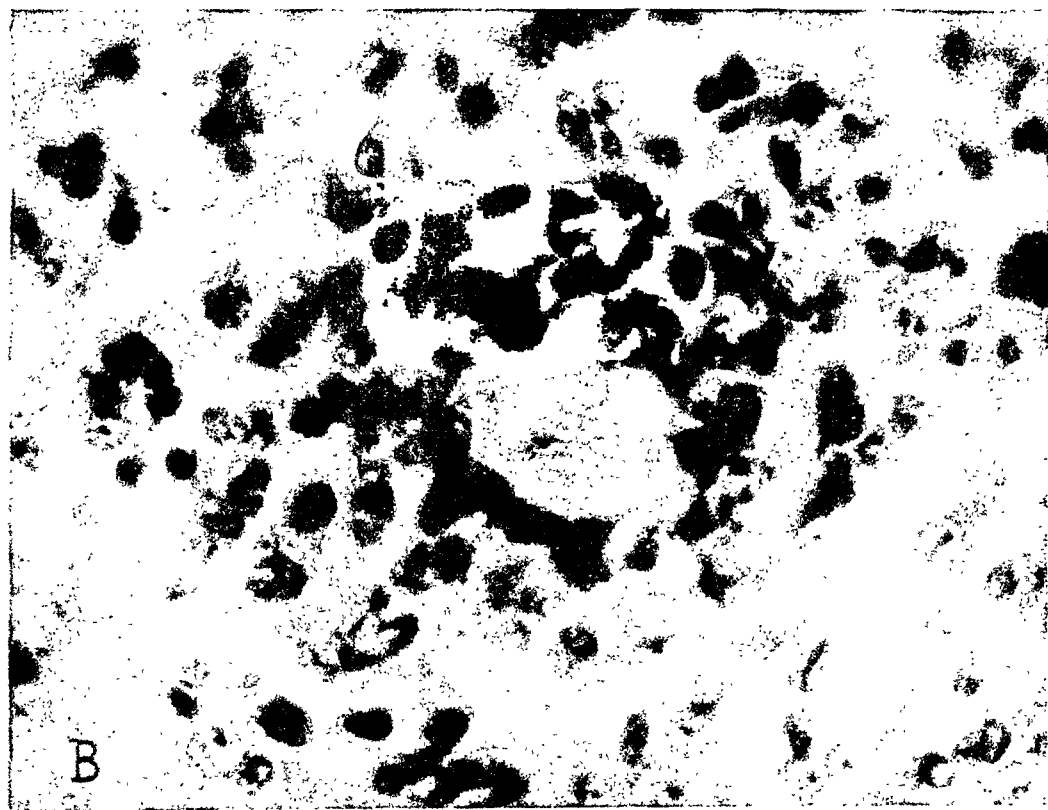
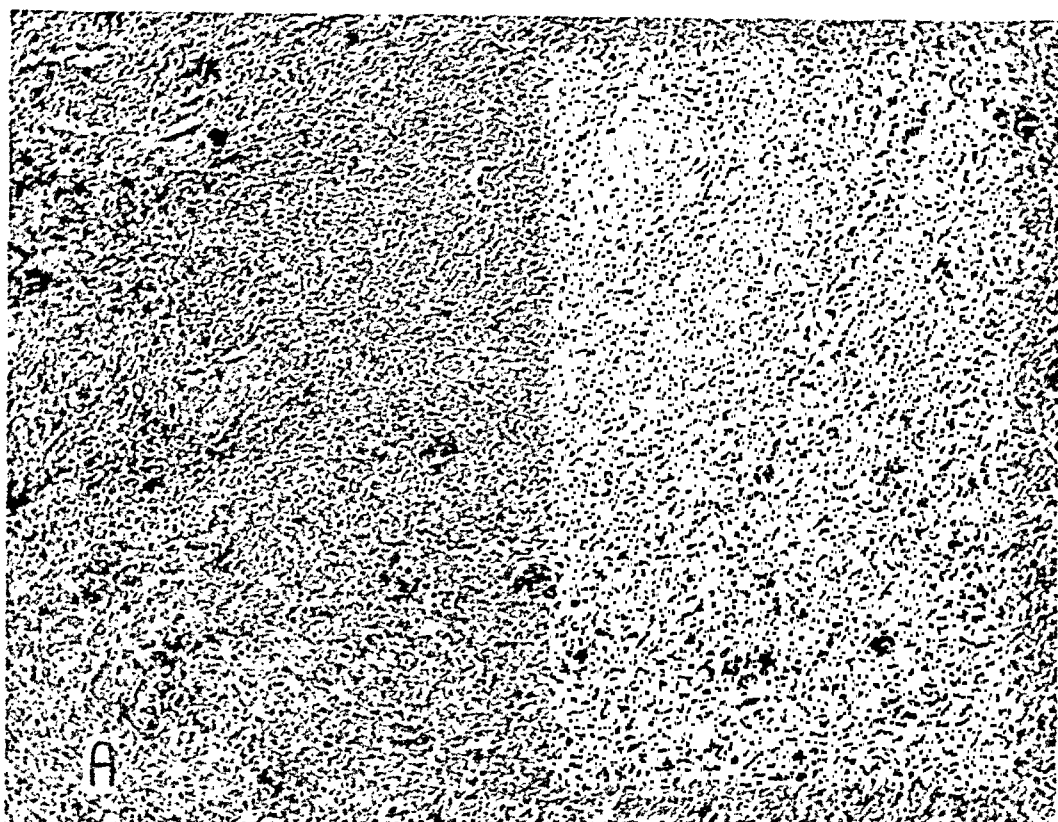


FIG. 2

the spleen, revealed any histologic evidence of extramedullary myeloid metaplasia.

Experience of others has clearly emphasized the importance of symptomatic therapy, once the diagnosis is established. Splenectomy or roentgen irradiation has proved to be contraindicated, as the patients invariably died from three months to two years following such treatment. A splenectomy in our patient was also followed by a fatal result. Our reasons for suggesting splenectomy were the patient's lack of improvement from repeated blood transfusions, her repeated and continuous blood destruction and the difficulty encountered in finding compatible blood. It was believed that benefit might result from removal of the factor (the spleen) which was interpreted as causing blood destruction, or possibly an interference or suppression (release)⁶ of the red cells in the bone marrow (hypersplenism).¹ Our experience in this case, however, substantiates the reports of other investigators, that splenectomy in agnogenic myeloid metaplasia of the spleen is contraindicated.

Since aplastic anemia has been diagnosed clinically in some of the reported cases of agnogenic myeloid metaplasia, we should like to direct attention to the comment made by Rhoads⁹ in his discussion on aplastic anemia. He stated that no form of treatment has been of any avail, that transfusions have had only a temporary effect and, indeed, at certain stages seems to have been actually harmful, leading to acute hemolytic episodes. Furthermore, those patients given roentgen radiation as a therapeutic measure have reacted very badly. In fact, many cases have been encountered in which refractory anemia has been the only diagnosis possible by any clinical or histologic criteria. The cases were observed for long periods, and as a preterminal feature unequivocal evidence of leukemia developed. The suggestion is strong that certain types of anemia may well be considered to be preleukemic conditions. From the experience of Rhoads in the study of aplastic anemias, one sees how care must be exercised in the differential diagnosis of agnogenic myeloid metaplasia of the spleen from aplastic anemia and from leukemia. In the early reports of agnogenic myeloid metaplasia of the spleen, these diagnoses have been frequently encountered. In the latter conditions, however, when repeated bone marrow studies fail to reveal pathologic changes characteristic of leukemia, then one must strongly consider this diagnosis. It is not only important that the diagnosis be properly established, but it must be remembered that, in this condition, splenectomy is definitely contraindicated.

There are other factors of pathologic and clinical interest in our case. The patient developed hemosiderosis and jaundice, which we interpreted on the basis of severe blood destruction following her repeated and numerous blood transfusions. The reports in the literature of jaundice and anemia in agnogenic myeloid metaplasia further indicate that continued blood destruction and hemolytic jaundice are part of this syndrome. Furthermore, diabetes mellitus de-

FIG. 3A. Section of bone marrow showing marked cellularity with frequent megakaryocytes, reticulum cells and macrophages. $\times 400$.

FIG. 3B. Smear of bone marrow (sternal puncture) showing a macrophage surrounded by myeloid cells. $\times 1100$.

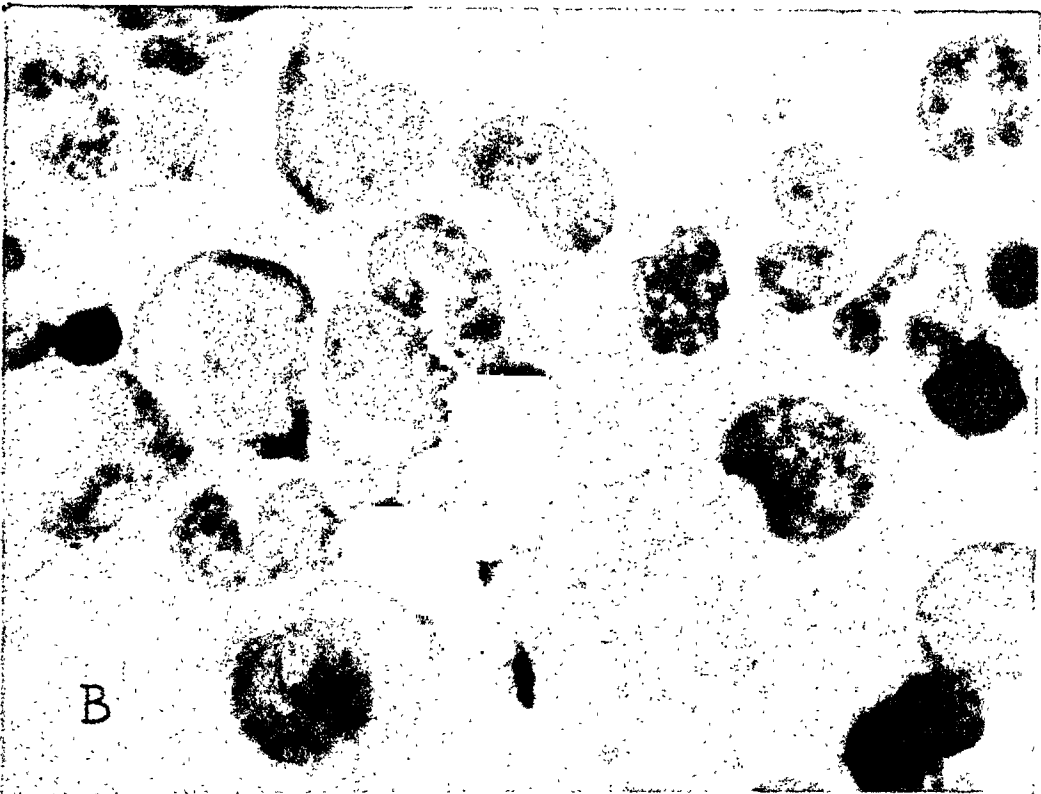
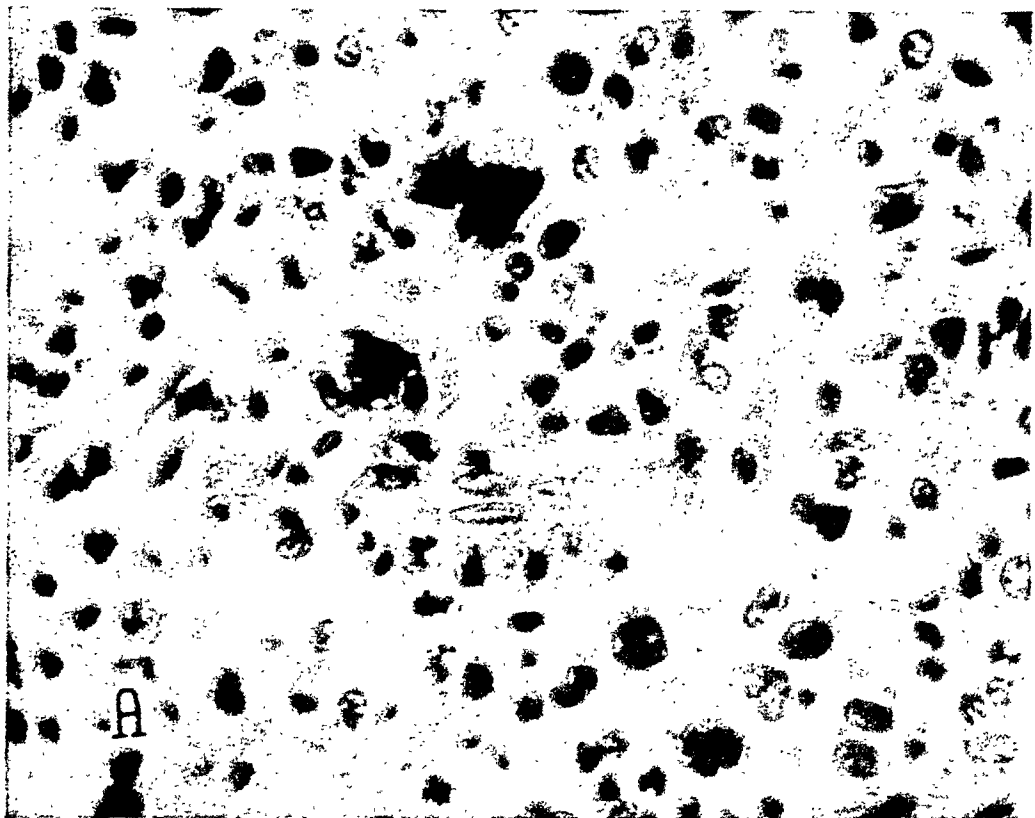


FIG. 3

veloped in our patient, and we were able to demonstrate deposits of hemosiderin in practically all of the organs, particularly in the pancreas and especially in the islands of Langerhans. The desposition of this pigment in the islets obviously interfered with insulin production.

Following splenectomy, our patient developed a chronic abscess in the left upper quadrant of the abdomen, a subacute fibrinous pericarditis and septicemia (*Staphylococcus aureus*). These are factors that hastened the demise of the patient.

As in agnogenic myeloid metaplasia of the spleen, the cause of most blood dyscrasias is not known. Recently, much thought has been given to the study of volatile organic chemicals in association with the anemias and leukemias. Mallory and his associates⁷ have presented histologic material from patients with a history of chronic exposure to benzene. They have shown that not merely the bone marrow, but the entire hematopoietic system, characteristically shows change. The bone marrow picture may vary from hypoplasia to the most extreme hyperplasia and extramedullary hematopoiesis, and in two of their cases this was associated with typical leukemic patterns.

SUMMARY

A case of agnogenic myeloid metaplasia of the spleen which was diagnosed clinically, is reported with necropsy findings.

The essential clinical findings of this condition are insidious onset of progressive weakness, abdominal distress and enlargement of the spleen, with or without hemorrhagic tendency. The hematologic findings are characterized by a slightly elevated leukocyte count with the presence of immature red and white elements in the peripheral blood. A biopsy of bone marrow may show normal, hypoplastic, or hyperplastic marrow with marked myeloid immaturity and no involvement of the myeloblastic tissue. The megakaryocytes are increased and present a normal dispersion.

The spleen is enlarged and, histologically, shows fibrosis and myeloid metaplasia. In our patient there was no evidence of myeloid metaplasia in any of the organs other than the spleen.

Our experience in this case substantiates the reports of other investigators that in agnogenic myeloid metaplasia of the spleen splenectomy or roentgen radiation of the spleen are contraindicated.

ADDENDUM

Dr. Paul R. Cannon, chairman, Department of Pathology, University of Chicago, has kindly given us permission to add to our presentation a case of agnogenic myeloid metaplasia of the spleen that he has studied. His patient was a white female, 48 years of age, with a spleno-hepatomegaly. Her peripheral blood showed a macrocytic type of anemia and a leukemoid blood picture; the sternal marrow showed a normal cellularity. The clinical diagnosis was Banti's syndrome. The spleen was removed and the histologic diagnosis was agnogenic myeloid metaplasia. The patient expired two months following splenectomy. The liver showed extramedullary hemopoiesis.

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A SURVEY OF COMMERCIAL Rh ANTISERUMS*

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The increasing recognition of the importance of the Rh factor in medical practice has taken the testing for this blood factor out of the hands of the specialist and placed it in the realm of the medical technician in the hospital and in the doctor's office. Despite the marketing of diagnostic antiserum by numerous commercial laboratories, the demand for such antiserum has been so great that it is often difficult to obtain any for long periods. Such a situation must inevitably lead to some carelessness in production unless there is some central control of the assay, labeling and standardization of Rh antiserum.

We were impressed with the problem when we began a survey for the presence of the Rh factor in pregnant women at the White Memorial Hospital, Los Angeles. The varying quality of different serums soon became obvious. Before beginning our main study, a comparison of various commercial antisera seemed necessary.

EXPERIMENTAL

Six serums from four different manufacturers were tested. Product A, a guinea pig serum, was used for the rapid slide agglutination test. The others were antisera of human origin used in the test tube method.

The tests were run according to the instructions of the manufacturers of the serums. Product A was tested against a 10 per cent red blood cell suspension; the others, against a 2 per cent suspension. All test tube trials were run as follows:

1. One drop of the anti-Rh serum was mixed with one drop of a washed 2 per cent blood cell suspension.
2. These were incubated at 37 C. for from thirty minutes to one hour, according to the individual instructions.
3. The tests were then read macroscopically, centrifuged at 1000 R.P.M. for one minute, then read microscopically.

The results are listed in Table 1.

Product A, a guinea pig serum, proved of no value in the newborn and in infants up to one month of age, since all of them gave positive reactions. This is in accordance with the findings first described by Fisk and Foord.¹ In most cases, blood specimens were tested by more than one of the serums listed in the above table. The results with products D and E fell far below the expected 85 per cent. The results with products A, B and C, at first glance, might seem accurate within the range of statistical error. Products A and B gave 85 per cent positive findings and product C, 87 per cent positive results. Actually,

* Received for publication, February 7, 1947.

a detailed examination of the data demonstrated that this accuracy was only apparent and not real. Four bloods which were positive with product C were negative with B, and eight bloods which were negative with product C were positive with B. This distribution is obviously due to factors other than the mere variation between serums giving 85 and 87 per cent positive findings, respectively.

Comparison of the two "85 per cent serums", B and A, further substantiated the inaccuracy of the apparently accurate data. Six bloods which were positive with product A were negative with B and two bloods which were positive with B were negative with A.

TABLE 1
A COMPARISON OF DIFFERENT COMMERCIAL Rh TYPING SERUMS

COMMERCIAL SERUM	BLOOD USED	NUMBER OF SERUMS TESTED	PER CENT Rh-POSITIVE
Product A.....	Infant	95	100
	Adult	225	85.7
Product B.....	Adult and infant	176	88.7
Product C.....	Adult and infant	249	88.0
Product D			
Sample no. 1.....	Adult and infant	101	80.2
Sample no. 2.....	Adult and infant	44	27.3
Product E			
Concentrated.....	Adult and infant	179	44.7
Diluted according to instructions..	Adult and infant	30	10.0

Product E was accompanied by instructions for diluting the serum. The results are given for the diluted and undiluted serums. Products E and D were supposedly standard "85 per cent serums".

Titration of the antisera against Rh-positive cells revealed the following results:

Product B.....	1:4
Product C.....	1:8
Product D	
Sample 1.....	1:32
Sample 2.....	1:16
Product E.....	1:4

The titrations were run by the standard test tube method with the final dilution determined by the addition of the red blood cells. The points were read microscopically.

DISCUSSION

The variations which were present in the serums tested might have been due to a number of causes. First, deterioration might have occurred between the time the serum left the manufacturer and the time it reached the laboratory. All serums were used within the expiration dates set by the commercial laboratories. There was, however, no uniformity in the expiration periods which were

set. Products B and C came prepared in the form of dry powder and each had an expiration period of thirty days. Products E and A, which were undesiccated serums, had a three month and a six month period, respectively, before expiration.

Our own experience indicates that deterioration did occur. Obviously, more study is essential to determine the optimum conditions for preservation.

Another problem is the initial strength of the serum. Although there is no definite relation between the titer of the serum and the number of Rh-positive bloods in our figures presented above, nevertheless, the strength of the serum must play a rôle in the accuracy of the test. No data are given on any of the serums examined as to control titration of the product, although we have the written and verbal assurance of most laboratories that such assays are performed routinely.

The actual details of procedure might have accounted for some error. We followed instructions in each case. If the procedures required a more meticulous technic than that which we employed, we believe that the test was too sensitive for routine work. A more likely assumption is that our procedures were adequate. We must emphasize, however, that variation from this technic may lead to error. If the results are not read microscopically, as for example in the test tube method, accuracy decreases. Philip Levine² advises, contrary to the instructions of one commercial concern, that tests should be run only by qualified technicians and not by the physician himself in his spare time.

One final source of difficulty should be mentioned. This has to do with the terminology of the subgroups of the Rh factor. In this country, Wiener⁴ and Philip Levine³ lead two schools of thought on the correct genetic interpretation of the Rh phenomenon and the terminology of the subgroups. It is essential for the whole problem to be clarified so that the laboratory worker will know, at least, the practical aspects of the terminology. At the International Hematology and Rh Conference recently held in Dallas, Texas, most workers seemed to support the British terminology,² which is in accordance with Levine's theory.

To illustrate the confusion in the labeling of commercial serums, product C, an "87 per cent positive serum", is listed as anti-Rh₀' which certainly doesn't conform to any terminology. Taken literally, it is a serum against the Rh₀ and Rh' factors and does not include the Rh" group and, therefore, should fall short of giving 87 per cent positive findings.

CONCLUSION

Six different Rh-antiserums from four different manufacturers were tested on a series of bloods from patients. The results varied from 10 per cent to 100 per cent positive, instead of from the theoretical variation of 85 to 87 per cent. A definite need for standardization and control of Rh diagnostic antiserums is indicated.

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THE RÔLE OF A PRESUMED SERUM PROTEIN IN THE PATHOGENESIS OF ERYTHROBLASTOSIS FETALIS*

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The difficulty of finding Rh antibodies in individuals with clinical signs of Rh sensitization has prompted the search for new tests in their detection. The open slide test of Diamond and Abelson³ has led to the description of the "conglutination test" for Rh antibodies by Wiener.¹⁰ In the latter test, human serum or plasma is used instead of saline as a diluent for erythrocytes and for the serum to be examined. The conglutination test has proved superior to previously employed tests for the detection of Rh antibody. According to Wiener, this test is characterized by a two-stage reaction in which a serum component, presumed to be identical with the X-protein described by Pedersen,⁵ is responsible for the activation of the antigen-antibody reaction. Another method for the detection of Rh antibodies was proposed by Coombs, Mourant and Race.² In this test, the sensitized erythrocytes are agglutinated by an anti-human globulin serum. The same principles were employed by Hill and Haberman⁵ in their "developing test".

One of the puzzling features in the pathogenesis of hemolytic disease of the newborn is the fact that in a large number of cases, hemolytic manifestations become apparent only *after* delivery. In attempting to explain this phenomenon, Wiener postulated that a serum component, the X-protein, which is formed only soon after birth, initiates the hemolytic process in the newborn.^{10, 11, 12}

Boorman, Dodd and Morgan,¹ have observed that when human serum is used as a diluent in titration of immune A,B,M,N and Rh antibodies, it enhances the action of these antibodies. The results of Boorman and co-workers indicate that all human serums share the capacity to enhance the action of immune antibodies, although some variations in the degree of enhancement have been found. The property of serum to enhance the agglutination of immune antibodies appears to be the basis of the conglutination test.

We have endeavored to determine whether the property of serum to enhance the action of immune antibodies is present in fetal blood and in the blood of infants. A preliminary note on this subject was published elsewhere.⁹

MATERIALS AND METHODS

The following serums were used in the titration of the immune antibodies: Two anti-Rh serums with a titer of 1:256 and 1:64, respectively, obtained from two Rh-negative women delivered of erythroblastotic babies; one anti-A serum, having a titer of 1:256,000, obtained from a pregnant woman suffering from Gaucher's disease; and one anti-B-serum with a titer of 1:2048, obtained from a

*Received for publication, February 24, 1947.

woman of group A who delivered a healthy child of group B. The titers cited above were obtained by the agglutination method.

The four serums were examined for agglutinating antibodies in serial dilutions in normal saline. Titration of these serums was also made by the agglutination method, using as diluent adult plasma of the AB group, and different compatible

TABLE 1
RESULTS OF TITRATION OF IMMUNE ANTIBODIES IN 93 HUMAN SERUMS

SOURCE OF SERUMS USED AS DILUENTS FOR IMMUNE SERUMS AND SUSPENSION OF ERYTHROCYTES	ANTI-RH SERUM AGGLU- TINATION TITER 1:8 (CONGLUTINATION TITER 1:64)	ANTI-RH SERUM AGGLUTINATION TITER 1:128 (CONGLUTI- NATION TITER 1:256)	ANTI-A SERUM AGGLUTINA- TION TITER 1:32,000 (CONGLUTINATION TITER 1:256,000)	ANTI-B SERUM AGGLUTINATION TITER 1:128 (CONGLUTINATION TITER 1:2048)
Umbilical blood (41 cases)	1:1 (2 cases) 1:2± (3 cases) 1:2 (7 cases) 1:4± (4 cases) 1:4 (8 cases) 1:8 (3 cases)	1:32 1:64 (2 cases) 1:256± (3 cases)	1:8000 (2 cases) 1:32,000 (2 cases)	1:16 1:64 (2 cases) 1:128
11 Children up to 6 months of age	1:4± 1:4 1:8± 1:8 1:32		1:2000 (2 cases) 1:4000 1:16,000 (2 cases)	1:64
10 Children from 6 to 18 months of age	1:8 1:32 1:64± 1:64 (2 cases)	1:256 (2 cases)	1:128,000 1:256,000 (2 cases)	
13 Pregnant women	1:32-1:64 (10 cases)			1:1024-1:2048 (3 cases)
13 Adults	1:64 (11 cases)			1:1024-1:2048 (2 cases)
5 Adults: diagnoses				
Infectious hepatitis	1:64			
Cirrhosis of liver	1:32 (2 cases)			
Cholecystitis	1:64			
Hepatitis	1:64			

serums from persons of various age groups. There were 41 serums of cord blood from normal babies, 11 serums of infants up to the age of 6 months, 10 serums of infants aged from 6 to 18 months, 13 serums of healthy adult males and females, 13 serums of pregnant women and 5 serums from patients suffering from liver disease. The serums of the adults served as controls. The method of titration was as follows. Three rows of small agglutination tubes were arranged. To each tube, 0.2 cc. of the respective diluent was added. Normal saline was the diluent used in the first row, adult AB plasma in the second row and the serums

to be examined in the third row. Two-tenths (0.2) cc. of the serum containing the antibodies was added to the first tube in each row. By transferring 0.2 cc. from the mixture in the first tube to the second tube, 0.2 cc. from the second tube to the third tube, and so on, double serial dilutions were made. A fresh pipet was used for each row of tubes. A 2 per cent erythrocyte suspension was prepared in the respective diluents for each row, *i.e.*, for the first row in normal saline, for the second row in AB adult plasma and for the third row in the serum to be examined. Type O Rh-positive blood was used for the titration of the Rh anti-serums, blood group A erythrocytes for the anti-A serum and B blood for the anti-B serum. The results of the agglutinations were read for the Rh-antiserums after one hour in a water bath at 37 C. and for the A and B antiserums after one-half hour at room temperature. The last tube with microscopically visible clumping was considered as the end titer.

TABLE 2

SUMMARY OF TABLE 1, SHOWING ENHANCEMENT OF IMMUNE ANTIBODIES BY VARIOUS HUMAN SERUMS

SOURCE OF SERUM	NUMBER OF SERUMS EXAMINED	NUMBER OF "NONENHANCING" SERUMS	NUMBER OF "ENHANCING" SERUMS
Umbilical blood	41	38	3
Infants 1 to 6 months	11	10	1
Infants 6 to 18 months	10	1	9
Persons 17 to 40 years	13		13
Pregnant women	13		13
Adults with hepatic diseases	5		5

RESULTS AND DISCUSSION

The results of titrations are tabulated in Table 1. In Table 2, the results have been summarized according to the reaction of serums with respect to the "enhancing" property. A serum was regarded as having enhancing properties if it reacted, in titration, like mature serum. By the term "nonenhancing" serum, we refer to serum which reacted in a manner similar to saline when used as a diluent.

The results of our investigations corroborate the observations made by Boorman, Dodd and Morgan¹ on the enhancing properties of mature human serums on the agglutination titers of immune antibodies. We were able to establish that these properties are not influenced by pregnancy or by pathologic conditions of the liver (cirrhosis and hepatitis). This enhancing property, however, is usually not found in cord blood or in the blood of infants up to 6 months of age since, out of 52 cases, only 4 behaved like mature serums. The identical effects of mature human serums on various immune antibodies indicate that the validity of the conglutination reaction is not limited to Rh antibodies.

Our investigation began with the question of the rôle played by a serum factor, X-protein, in the pathogenesis of erythroblastosis fetalis. According to Wiener, fetal blood lacks this X-protein, which is presumably formed in the newborn soon after delivery. The presence of this factor initiates the hemolytic disease in the apparently healthy child. This would imply that the presumed X-pro-

teins precipitate *in vivo*, a process similar to that taking place in the conglutination reaction. From the results of our study on cord bloods of normal babies and of serums of infants up to the age of 6 months, it is evident that they do not possess enhancing properties in the agglutination reaction of immune antibodies. Although we had no opportunity of examining serums of erythroblastotic children as to their enhancing property on immune antibodies, it is not reasonable to assume that only serums of erythroblastotic children may contain the presumed X-protein. Diamond and Denton⁴ and Levine and Bernstein,⁷ reported successful enhancement of immune antibodies by a great variety of substances, especially those containing protein. We may accept the view that this enhancing property in mature human serum is due to some serum protein. Should we presume that this serum protein is able to cross from the maternal to the fetal circulation, it would still be difficult to explain the postnatal appearance of hemolytic manifestations in most cases of erythroblastosis fetalis.

From the results reported here, it would appear difficult to ascribe any significance to certain serum properties in explaining the occurrence of erythroblastosis fetalis in babies soon after delivery.

SUMMARY

Serums from umbilical blood, infants and adults, have been studied to ascertain their enhancing properties on various immune antibodies.

Umbilical serums and serums of infants up to the age of 6 months were found to be devoid of enhancing properties.

Serums of infants aged 6 months and over, as well as serums of healthy adults, of pregnant women, and of patients with hepatic disorders, have all been found to enhance the action of various immune antibodies.

It may be concluded that there is no evidence for the presumed rôle of a so-called serum factor or X-protein in the pathogenesis of hemolytic disease of the newborn.

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NONPRODUCTION BY PENICILLIN OF FALSE POSITIVE REACTIONS IN KAHN AND KOLMER TESTS*

RALPH D. TURNER

When, in 1943, Mahoney and his associates³ announced that penicillin was efficacious in the treatment of syphilis, and subsequently with the intensive therapeutic use of penicillin, it became desirable to determine the effect of penicillin on the serologic tests for syphilis.

In the summer of 1943, these investigations were begun in the Department of Serology, of the Naval Medical School, Bethesda, Maryland. Inasmuch as it had been shown that the actions of penicillin *in vitro* and *in vivo*¹ were apparently not the same, it was reasoned that the effect on the Kahn and Kolmer tests of penicillin added to solutions used in performing those tests might differ from the effect on serums which already contained penicillin as a result of the therapeutic use of the drug. It was thought by some that the injection of penicillin into patients resulted in a by-product which had a part in the action of penicillin therapy and might affect serologic tests.

The following investigations and observations were made.

1. Fresh penicillin solution was added to the saline used in performing the Kahn tests (Presumptive, Standard and Quantitative) in concentrations found in patients treated with penicillin (varying number of Oxford units per cc.). Tests were run on serums of syphilitic subjects and of normal, negative, non-syphilitic volunteers. No changes in the titers of the serums of the syphilitic patients were noted and no false positive reactions in the serums of the other persons were seen.

2. Fresh penicillin solutions (in concentrations ordinarily equal to, and greater than, those found in the blood) were used in duplicate titrations for the Kolmer tests and also in duplicate Kolmer Qualitative and Kolmer Quantitative tests with no effect upon these tests.

3. Kahn and Kolmer tests, with and without penicillin added, were performed on the serums of nonsyphilitic patients who were receiving penicillin therapy, chiefly because of staphylococcic or streptococcic infections. No positive Kahn or Kolmer reactions were obtained in this group.

4. Very strong dilutions of penicillin were found to be slightly anticomplementary in experimental titrations (Kolmer), but it is most unlikely that such concentrations would ever be encountered in routine work.

SUMMARY

In tests made on 146 serums to which penicillin was added, no false positive Kahn and Kolmer tests were obtained. It is believed that the therapeutic

* Received for publication, March 12, 1947.

use of penicillin can be discounted as a source of false positive serologic tests for syphilis.*

Acknowledgments. The author wishes to express his appreciation to Capt. Paul W. Wilson, MC, USN, Commanding Officer, and to Capt. O. Wildman, MC, USN, Director of Laboratories, Naval Medical School, Bethesda, Maryland, for permission to conduct these studies; to Comdr. E. E. Barksdale, MC, USNR, and Comdr. A. M. Hutter, MC, USNR, of the Naval Hospital, Bethesda, Maryland, for supplying blood specimens and necessary clinical data; and to Lt. Comdr. Paul Woolley, MC, USNR, for making the penicillin blood level determinations.

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* This finding concurs with the experience of Dr. John A. Kolmer.²

SOME CLINICAL OBSERVATIONS ON THE PROTHROMBIN TEST*

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The prothrombin test has become clinically useful with the advent of anti-coagulant therapy. There is, however, still a great deal of confusion concerning the indications for this test and the interpretation of its results. This is especially true of the reports by various authors on studies using Dicumarol (3, 3'-methylene-bis-[4-hydroxycoumarin]) as an anticoagulant *in vivo*. The necessity for less ambiguity in the interpretation of the prothrombin test has been stressed in a previous communication.⁴

Indications for the use of the prothrombin test fall into three groups: in differentiating bleeding states due to hypoprothrombinemia from those caused by other conditions; as a diagnostic aid in the differentiation of obstructive jaundice (stone or stricture of the ductus choledochus); and in following the course of therapy with Dicumarol. The prothrombin test should not be used as a routine liver function test.

In the majority of reports in the literature concerning prothrombin levels, the results of the test are given in terms of "seconds" or of "per cent", without any mention of the method used. In some instances, the prothrombin time of a "normal patient" is given. The use of normals, in this sense, is fallacious, as will be shown in this report. In reviewing the literature and testing the proposed methods, a great variation was seen in the type of procedure and also in the manner of expressing the results. Thus, there is no real standardization of technics or of prothrombin values, which may lead to confusion in connection with anti-coagulant therapy. While some of these methods can be used as a "relative index" of the prothrombin concentration, most of them are not accurate enough to follow that course of Dicumarol therapy. This is true of the so-called "bed-side" and "rapid" prothrombin methods, especially those using blood from a finger puncture.

The many suggested prothrombin methods and modifications can be divided into two large groups: the whole blood and the plasma methods. The latter can be further divided into those employing whole plasma and those employing diluted plasma. Each method will be discussed in turn.

According to Howell,⁷ prothrombin determinations are based on the following reactions: (1) Prothrombin + thromboplastin + Ca^{++} → thrombin, (2) Thrombin + fibrinogen → fibrin.

From these reactions, it is seen that if the thromboplastin, Ca^{++} , and fibrinogen are constant or controlled, the production of a fibrin clot can be used as the stoichiometric point in a quantitative titration, with the prothrombin as a

* Received for publication, March 26, 1947.

variable. In all of the methods this is based on a time factor: the time required for formation of the clot, after the proper constituents entering into the reaction have been mixed. This time must, of course, be converted into suitable equivalents to express the amount of prothrombin in the blood. To report the seconds required for the clot to form does not allow for the variability of the different methods or reagents.

WHOLE BLOOD METHODS

The whole blood methods are used because they are easy, and a minimum of equipment is needed. The simplest of these is the "slide test";¹² a drop of blood from a finger or an ear puncture is added to a drop of thromboplastin emulsion on a slide and the mixture is stirred, or a pin is drawn through it, until a fibrin clot forms. There are many variations of this procedure,^{1, 10} and all are subject to error in that the drop cannot be kept constant and the admixture of various amounts of tissue fluid cannot be avoided. This is important when working with so small an amount of blood and so complex a mechanism.

Smith's "bedside" one-stage test,¹⁶ which was first reported by Quick,¹² is performed in the same manner as the Lee-White test for coagulation time, with the addition of thromboplastin. It represents, then, a method for determining accelerated coagulation time rather than the prothrombin time. Smith's test is widely used in small hospitals and laboratories, but has disadvantages which will be discussed together with the errors of whole blood methods in general.

Another whole blood technic which has been used is Kato's microprothrombin method.⁷ It was developed for use primarily in pediatric practice since it uses blood from a finger or a heel puncture. The method, however, entails the use of special microhemopipets, which require some previous training in their manipulation before accurate results can be obtained. Kato stated that his method is but a procedure for determining an accelerated clotting time.⁹

It can be seen that there are many disadvantages and inaccuracies connected with the whole blood procedures. Since almost all of these technics use blood which is not treated with an anticoagulant, it is necessary to perform repeated venipunctures or finger tip punctures in order to carry out more than one determination. Accuracy of any degree cannot be obtained with a single determination on a whole blood specimen, since, as will be shown later, there is some degree of statistical deviation of a given sample even with more accurate methods. Multiple determinations (two or three) should be carried out, and their average taken, in order to procure values of greater accuracy.

Comment can be made on the two whole blood methods which are used more frequently (Smith's and Kato's), and on their variations. Equal volumes of the patient's and "normal" bloods are used, and a ratio of the relative amounts of prothrombin is assumed. This is sometimes expressed as "clotting power" or "prothrombin index". It must be remembered, however, that the prothrombin fraction of the blood is in the plasma, and that the red blood cells replace a certain volume of this plasma. This would not be significant were it not for the differ-

ence in hematocrit values of the patient and of the "normal". Thus, equal total volumes of whole blood do not always provide equal amounts of plasma and of red blood cells.

Although Abramson, Weinstein and White¹ stated that they consider blood to be 1:1 suspension of cells and plasma, it is erroneous to follow this formula. I have observed patients with portal cirrhosis who have given prothrombin values of over 100 per cent (compared with tests run on blood of "normal" individuals at the same time) by the Smith's bedside test, yet have given consistently low values (30 to 40 per cent) by Quick's method (Table 2). Patients with cirrhosis frequently show a secondary anemia and a low hematocrit, especially before any therapy has been instituted. These tests have been interpreted to mean that although equal volumes of whole blood were used, cirrhotic patients had more plasma per unit of whole blood than normal. Therefore, although they may have less prothrombin per unit of plasma, these patients have sufficient plasma to compensate when aliquot amounts of plasma are not used. Furthermore, it is difficult to judge the end point of the reaction, using whole blood, since the opaque color masks the clot formation. The sensitivity of these tests approximates that of clotting time methods, which are recorded in terms of half-minutes. The whole blood methods, are usually expressed in terms of the "normal" blood run at the same time; this makes the determination variable, depending on the hematocrit and prothrombin values of the "normal" used. The values are then represented by a linear curve, whereas serially diluted plasmas give a hyperbolic curve. Hauser⁵ and Quick¹⁴ have reported the occurrence of familial hypoprothrombinemia which was refractory to vitamin K. This increases the hazard of using different "normals" as a standard each time a determination is run. It is believed, therefore, that whole blood methods and this method of expression of prothrombin levels often have sources of errors.

PLASMA METHODS

Plasma prothrombin methods are believed to be more accurate, since aliquot portions of plasma are used and the prothrombin is contained in the plasma. These methods can be divided into "whole" and dilute plasma procedures, and considerable controversy has been waged over the sensitivity and accuracy of one method as opposed to another. It should be remembered that a single prothrombin level has no more significance than a single determination of the basal metabolic rate; it is best to follow the patient over a short period of time and notice the trend of the prothrombin values procured.

DILUTED PLASMA

Brambel,³ Allen *et al.*,² Shapiro *et al.*,¹⁵ and several other investigators have proposed modifications of plasma prothrombin methods in which the plasma is diluted to 50 or 12.5 per cent before carrying out the procedure. They claim that these dilutions increase the sensitivity of the test by increasing the time of formation of the clot. Therefore, they claim that differences in time of clot formation are more significant. These "sensitized" procedures have been the

basis for claims that xanthine and salicylate drugs produce hypoprothrombinemia and for the relationship of hyperprothrombinemia to thrombosis.

Plasma dilutions of the order of 10 to 12.5 per cent, made with 0.9 per cent saline solution, show great variability in clotting, since the fibrinogen and electrolyte concentrations are similarly reduced. The quality of the clot is also poor. It is not feasible for small laboratories to prepare, by adsorption methods, prothrombin-free plasma (which is an ideal diluent), since this would involve more time and work than is desirable for the preparation of reagents for a simple laboratory test. As is shown in Table 1, the standard deviation at 10 per cent is much greater than that at 50 to 100 per cent. Prothrombin levels of 60 to 100 per cent are usually not significant in whole-plasma methods since there is very little change in clotting time; levels below this (30 to 50 per cent which is the range concerned in Dicumarol therapy) are sufficiently prolonged to observe delays in clot formation. Therefore, there is no advantage in using diluted plasma in routine prothrombin determinations, since the dilution adds another source of error.

WHOLE PLASMA

The two whole plasma technics which are used are the one-stage method of Quick¹³ and the two-stage method of Warner, Brinkhous, and Smith.¹⁷ The two-stage method can be discussed briefly by stating that, although it is quite accurate as a medium in the study of the blood clotting mechanism and other complex research projects, it is beyond the scope of the average small hospital laboratory.

Quick's one-stage method has been shown to be an accurate and relatively simple procedure. It is accurate enough to follow Dicumarol therapy, and can be learned in a short time. By this method, serial dilutions of plasma (using 0.9 per cent saline or prothrombin-free plasma), are plotted to produce a hyperbolic curve; the concentrations of prothrombin being plotted on the abscissa, and the clotting times, in seconds, on the ordinate. Quick has calculated the following formula to fit this curve:

$$\% \text{ of prothrombin} = \frac{302}{\text{P.T.} - 8.7}$$

in which 302 and 8.7 are constants and P.T. is the patient's prothrombin time in seconds. One hundred per cent is the value of prothrombin represented by plasma which will clot in eleven to thirteen seconds, using an active thromboplastin preparation.

Much work has been done on the preparation of a suitable thromboplastic substance for use in the prothrombin test. Rabbit and beef lungs have been used as sources of the material, as well as rabbit brain (acetone-dehydrated according to the method of Quick). Fullerton⁵ and Page and Russell¹¹ have used Russell's viper venom, which shows thromboplastic activity, and a good degree of stability in the crystalline state. We have obtained good results with the product from rabbit brain prepared by Difco.

LABORATORY STUDIES

Three laboratory studies are included in this report. For all of the studies, blood was drawn by venipuncture and oxalated in the proportion of 0.1 cc. of 0.1 M sodium oxalate to 0.9 cc. blood. The specimens were centrifuged for fifteen minutes at moderate speed, and the supernatant plasma drawn off for use in the tests. Thromboplastin emulsion was prepared by incubating one ampule

TABLE 1

STANDARD DEVIATION OF PROTHROMBIN READINGS BY QUICK'S METHOD IN RELATION TO PERCENTAGE OF PROTHROMBIN

PER CENT OF PROTHROMBIN IN DILUTED PLASMA	PROTHROMBIN TIME IN SECONDS	STANDARD DEVIATION
10	74.2	± 5.3
20	37.5	± 3.8
30	27.1	± 2.9
40	22.1	± 2.0
50	19.0	± 1.6
100	14.4	± 0.9

TABLE 2

COMPARISON OF SMITH'S AND QUICK'S METHODS OF PROTHROMBIN TESTS IN 5 PATIENTS WITH PORTAL CIRRHOSIS AND IN 3 "NORMAL" INDIVIDUALS

PATIENT	SMITH'S METHOD		QUICK'S METHOD		HEMATOCRIT, PER CENT OF RED CELLS
	Reading in Seconds	Per Cent of Normal	Reading in Seconds	Per Cent of Prothrombin	
*N. B.	23.8	100.0	14.2	94.3	
O. L.	21.4	111.2	21.1	29.9	
*R. B.	20.5	100.0	14.0	100.0	54.0
F. W.	17.0	120.6	26.4	19.6	44.0
*E. L.	25.8	100.0	16.9	51.2	48.0
M. K.	24.0	107.5	19.8	34.3	48.0
A. G.	28.9	89.3	16.1	59.2	41.0
J. L.	24.6	104.9	19.3	36.4	34.0

* Represents "normal" subject.

(0.15 gm.) of Difco acetone-dehydrated rabbit brain with 2.5 cc. of oxalate-saline mixture, (90 cc. of 0.9 per cent saline solution and 9 cc. of 0.1 M sodium oxalate) in a water bath from twelve to fifteen minutes at from 45 to 48 C. The emulsion was centrifuged slowly for three minutes to settle the large particles, and then decanted through a small piece of cotton. The concentration of calcium chloride used to re-calcify the plasma was 0.025 M.

1. Ten "normal" patients, free from liver disease or blood dyscrasias, were studied. Five of them were men and five were women; two were negro and eight were white. The plasmas were diluted with 0.9 per cent sodium chloride solution to provide prothrombin concentrations of 100, 50, 40, 30, 20 and 10 per

cent. Three determinations, by Quick's method, were performed at each dilution, on each plasma sample. When plotted, these values gave the characteristic hyperbolic curve. Using the formula, the standard deviation at each dilution

$$\sigma_D = \sqrt{\frac{\sum(x^2)}{N}}$$

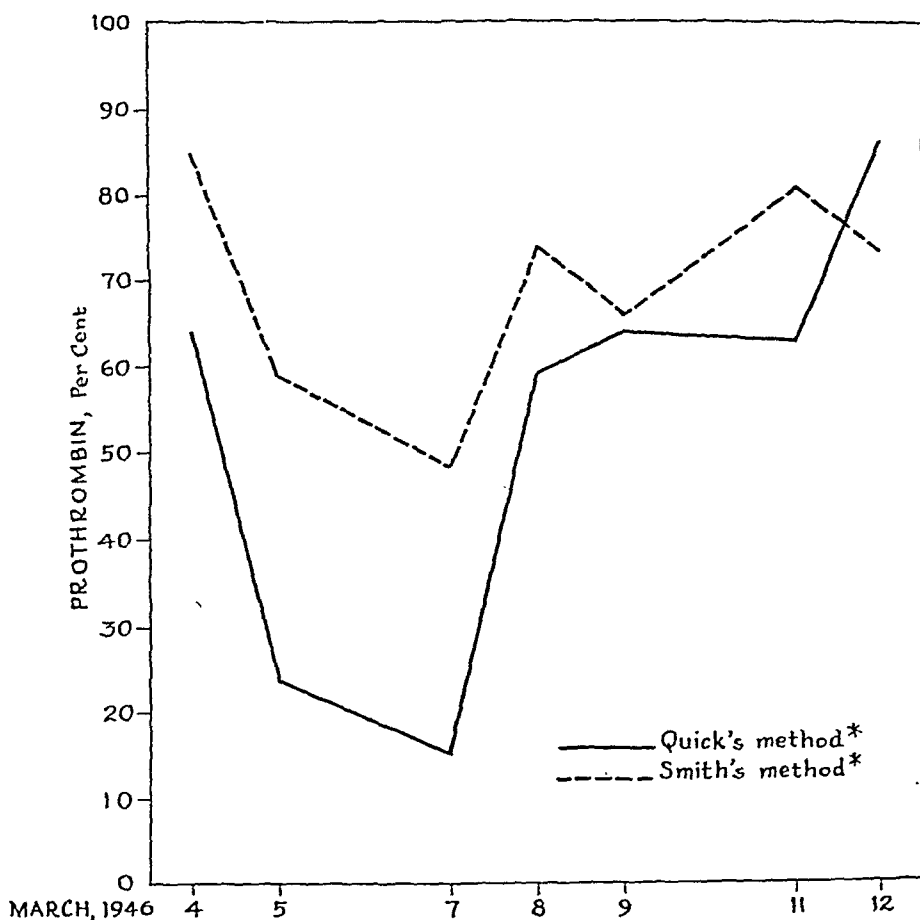


FIG. 1. Comparison of curves for Prothrombin Time as determined by Quick's and Smith's methods in a patient with postoperative pulmonary embolism who received Dicumarol therapy.

* Quick's Method: 13 seconds = 100 per cent of prothrombin

* Smith's Method: 17 seconds = 100 per cent of normal

Date	Amount of Drug Administered
3-3-46	200 mg. Dicumarol
3-4-46	300 mg. Dicumarol
3-5-46	50 mg. Dicumarol
3-7-46	5 mg. Vitamin K
3-9-46	50 mg. Dicumarol
3-10-46	50 mg. Dicumarol

was calculated for the series (Table 1). This study shows that the standard deviation increases in inverse proportion to the concentration of the plasma, and indicates the factor of "greater variability at low concentrations. When acknowledging the occurrence of 3σ , in 99 per cent of determinations, according to

the bell-shaped curve of distribution, it is seen that the deviation may be significant at the lower concentrations of plasma.

2. A second study concerned Quick's method using whole plasma and Smith's "bedside test" using whole, unoxalated blood. The values for Smith's test were computed as a percentage of "normal" blood which was tested at the same time:

$$\frac{\text{Prothrombin time of patient}}{\text{Time of normal}} \times 100 = \% \text{ of normal}$$

This is the usual calculation employed with the bedside and simple tests. For Quick's method, the percentages were computed using his formula. Since our 100 per cent values averaged to thirteen seconds, a correction for this was made in the formula:

$$\% \text{ of prothrombin} = \frac{302}{(\text{P.T.} - 2) - 9}$$

Five patients with a clinical diagnosis of portal cirrhosis were studied (Table 2). This study shows that considerably higher values are obtained using Smith's method. This finding is in keeping with the remarks made concerning whole-blood methods in the beginning of this paper.

3. The third study was made in a case involving Dicumarol therapy. A patient with suspected postoperative pulmonary embolism was seen on the obstetric service. She was given Dicumarol, and prothrombin tests were made by both Quick's and Smith's methods. The usual calculation for each method was made and compared (Fig. 1). At a few points in the plotted curve, the values for Smith's test followed those for Quick's method, although they were of a higher order; the trends were somewhat similar.

The Dicumarol was administered with relation to the values obtained by Quick's method, and was safely discontinued in ten days. At no time did the patient exhibit any hemorrhagic manifestations.

SUMMARY

A discussion of prothrombin tests and their values is presented. It is suggested that whole plasma be used in the performance of the prothrombin test rather than whole blood or diluted plasma since sources of error caused by dilution of the fibrinogen and electrolytes and by masking of the end point by whole blood, are obviated. Aliquot portions of whole plasma give a better means of comparison without the introduction of extraneous factors. It is also suggested that prothrombin levels be expressed in terms of per cent of prothrombin, as in Quick's method, rather than in seconds or in per cent of prothrombin time as determined in a "normal" subject.

Some laboratory studies are presented with a comparison of findings with Smith's "bedside test" and Quick's one-stage method.

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GENITO-URINARY INFECTION

BACTERIOLOGIC STUDY OF ONE HUNDRED FIFTY CASES*

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The following study is concerned with the isolation and identification of different species of organisms present in genito-urinary infections. It is presented primarily to acquaint workers with newer methods of culture and identification. The list of organisms so recoverable is broader than with earlier technics. To our knowledge, the best recent work in the field of genito-urinary bacteriology, in which modern cultural methods were used, was done by Schulte⁷ and Sandholzer and Scott.⁶

In our studies, specimens were taken at cystoscopy by staff members and resident physicians. The usual aseptic technic in collection was employed. Urine was obtained from the bladder, or the pelvis of the kidney as indicated, collected in sterile test tubes, provided with stoppers and sent to the bacteriology laboratory. The procedure then routinely consisted of centrifuging the specimens, staining the sediment and making a culture of the sediment on blood agar, plain nutrient agar, fluid thioglycollate anaerobic (Brewer's) medium, and on tryptose phosphate broth containing 0.5 per cent agar. The latter was incubated under increased CO₂ tension.

In instances in which growth was obtained, individual colonies were subcultured on Kligler's iron agar. Upon obtaining pure cultures on Kligler's medium, the organisms were stained by Gram's method and subcultures were made on tryptose-starch agar for pigment production, in nutrient broth and in a special motility medium. The latter consisted of 0.5 per cent agar in nutrient broth and was employed for motility studies. The presence of pleomorphism was noted.

The organisms were further studied routinely for reactions on the following carbohydrates: dextrose, maltose, mannite, sucrose, lactose, inulin, xylose; and on the glucoside, salicin. Reactions in additional carbohydrates and other special mediums were employed when necessary for complete identification of the organism. The carbohydrates were employed in 1 per cent solution in nutrient broth. The tubes contained inner fermentation tubes, which facilitated a high degree of accuracy in gauging the production of gas. Phenol red was used as the indicator except in certain special mediums. With organisms which produced acid and gas in lactose, additional subcultures were made in tryptophane broth for indol production, and in MR-VP (Difco) medium for the methyl-red and Voges-Proskauer tests. Gelatin liquification studies were done routinely on all organisms.

Comparative studies were made with several organisms, using both lead acetate agar and Kligler's iron agar for determining hydrogen sulfide production. Fur-

* Aided by Kass Fellowship Award, University of Texas Medical School. Received for publication, March 14, 1947.

ther comparative studies were made with several species, using indicator agar in parallel with the above mentioned indicator broth. Special mediums were employed for the *Mycobacterium* group when clinically indicated. These mediums were Harold's and Petragnani's medium. A comparatively new medium was utilized routinely in the last 100 positive cases to determine which organisms produced ammonia from urea. This medium (BBL) is easily made up. Its sterilization, by autoclaving at eight pounds of pressure for twenty minutes, proved satisfactory while the recommended filtration method is very cumbersome.

All streptococci were cultured in 6.5 per cent sodium chloride solution, in tryptose broth at both 10 C. and 37 C., at pH of 9.3 and in 0.1 per cent methylene blue-litmus milk. Some strains were checked by the Lancefield grouping method.

After complete identification as to genus and species, the strains were kept in stock on Avery's medium for possible future reference. The organisms were classified according to Bergey.¹

The following table gives the number of organisms isolated according to genus:

TABLE 1
ORGANISMS ISOLATED ACCORDING TO GENUS

<i>Staphylococcus</i>	41
<i>Aerobacter</i>	24
<i>Escherichia</i>	21
<i>Streptococcus</i>	21
<i>Alkaligenes</i>	14
<i>Eberthella</i>	8
<i>Pseudomonas</i>	7
<i>Proteus</i>	4
<i>Salmonella</i>	2
<i>Mycobacterium</i>	2
<i>Corynebacterium</i>	2
<i>Gaffkya</i>	2
<i>Shigella</i>	1
(<i>Saccharomyces</i>)	1)

Of the *Staphylococcus* group, 4 were pigment producers and 37 were not; 5 were beta-hemolytic and 36 nonhemolytic. *Streptococcus pyogenes* (Group A Lancefield) constituted 2 of the 21 identified and the remaining 19 were *Streptococcus faecalis*.

Notation was made of the "slow lactose fermenters", the so-called "paracolon" group of organisms and all those observed in this study fell into either the *Escherichia* group (6) or the *Aerobacter* group (5). It is interesting to note that all but one of the urea splitters of the *Aerobacter* group fall into this classification.

No urea splitting organisms were found in genera *Escherichia*, *Pseudomonas*, *Eberthella*, *Corynebacterium* and *Gaffkya*. This contrasts with Hill's report of finding one strain of *Escherichia* responsible for splitting of urea. Renault⁵

stated that organisms which do not liquefy gelatine do not break down urea. We were unable to substantiate this claim as we have encountered many organisms of the genus *Aerobacter* which did not liquefy gelatine, but which did split urea, and many gelatine liquefiers in the *Staphylococcus* group which failed to break down urea.

The following organisms in the series studied were found to split urea in the designated number of instances:

<i>Streptococcus</i>	12
<i>Staphylococcus</i>	10
<i>Aerobacter</i>	6
<i>Proteus</i>	4
<i>Alkaligenes</i>	3

This constitutes roughly about one-half of the streptococci, one-half of the aerobacters, one-third of the staphylococci, one-third of the alkaligenes and all of the protei organisms tested which were found to be urea splitters.

SUMMARY

The results of a bacteriologic survey of genito-urinary infections are presented. A comparative study was made of standard mediums used in modern bacteriologic laboratories for culturing bacteria found in genito-urinary infections. It is concluded that the best single medium is Brewer's fluid thioglycollate anaerobic medium and that the best combination of mediums is Brewer's medium and blood agar plates. Most strains for streptococci can be isolated on Brewer's medium, whereas many do not grow on blood agar or other aerobic mediums, although they are aerobic or facultative anaerobic strains. After initial growth in Brewer's medium, however, these grow readily on blood and other aerobic mediums, a not uncommon phenomenon. Brewer's medium is excellent for the isolation of aerobes as well as anaerobes; no organisms were isolated aerobically which did not also grow in Brewer's.

A study of BBL urea medium was made and it was found that many organisms which are not credited with breaking down urea with formation of ammonia readily do. In view of the urea fermentation theories of Creevy³ and of Chute,² this may prove to be of aid in the study of the formation of renal calculi.

The solid motility medium was found to be less confusing than the hanging drop for motility tests.

Petragnani's medium for the *Mycobacterium* group proved superior in this limited series. Broader use in all fields of bacteriology, however, proves that with it profuse growth may be obtained within ten days and all cases studied have checked completely with guinea pig inoculation. Since it inhibits contaminating organisms, it is a convenient medium for culture of contaminated specimens such as sputum.

Kligler's iron agar proved superior to lead acetate agar in determining hydrogen sulfide production.

Indicator broth was found to be more efficient than the indicator agar in cases

where a small amount of gas was produced, due to the difficulty in reading the results of gas formation in the agar.

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LEUKOCYTE COUNTS IN NORMAL WHITE WOMEN BEFORE AND AFTER DELIVERY*

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Leukocytosis occurring during pregnancy and labor may require evaluation in attempting to ascertain the possibility of a concurrent acute infection. One of us saw a patient in the first stage of labor, in whom the diagnosis of an acute surgical condition of the abdomen was made. A blood count at this time was difficult to interpret.

Despite frequent references in the literature to physiologic leukocytosis of pregnancy, there is considerable variation in the values reported. The leukocyte count during early labor ranges from an average of 7100 white blood cells per cu. mm., as reported by Boyd, Blenkinsop and Mylks,² to an average of 18,255 cells per cu. mm. as recorded by Baer.¹ Wolff,⁵ in a study of the leukocyte counts of 50 normal pregnant women, reported an average of 11,456 cells per cu. mm. during labor, when the pains occurred every five minutes and lasted from thirty to seventy seconds, and an average count of 15,150 white blood cells per cu. mm. at the onset of the second stage of labor. Carey and Litzenberg³ found that during pregnancy counts rarely exceeded 12,500 cells per cu. mm. They reported no uniform rise in the ninth month, maintaining that in some patients the leukocyte counts remained consistently low, and in others, consistently high throughout the nine months.

Baer,¹ who reported an average leukocytosis of 18,255 during labor, stated that the Arneth's index showed a displacement to the left. In DeLee's textbook, *The Principles of Obstetrics*, reference is made to an increase in the white blood cell count during pregnancy with an increase in the younger cells so that the blood picture resembles that in an acute infection. Heyn⁴ reported two leukocyte counts that were made two minutes and thirty minutes, respectively, before delivery. His findings are presented in Table 1.

In order to facilitate the interpretation of the leukocyte count in labor, a series of total and differential counts of the white blood cells was studied in normal pregnancies. Patients in the Druid City Hospital, Tuscaloosa, Alabama, were observed over a period of four months, from November 1941 to February 1942, inclusive.

METHODS

The following rules were adopted for selecting the subjects.

1. There shall be no abnormal physical findings, such as fever, heart murmurs, ankle edema, albuminuria, or abnormal blood pressure (normal values, systolic 130-100, diastolic 80-65).

* Received for publication, March 17, 1947.

2. The onset of labor must be spontaneous and the duration of labor shall not exceed thirty-six hours.

3. The gestational period shall not be less than two hundred and twenty days after the beginning of the last menstrual period.

4. There shall be no history of complications during previous pregnancies or of complications during the present gestation.

5. The leukocyte count shall not exceed 35,000 and the erythrocyte count must be at least 3,000,000 per cu. mm., with at least 60 per cent or 10.2 gm. hemoglobin (100 per cent equaling 17 gm.).

6. The amount of bleeding in the third stage of labor shall not exceed 600 cc.

In accordance with the above rules, 57 white patients were chosen, ranging in age from 17 to 42 years. Nineteen of the 57 patients were primigravidas, 21 had each delivered one child, 6 had each delivered two, 6 had each delivered three, and 5 had each delivered four or more normal offspring.

TABLE 1

LEUKOCYTE COUNTS IN 22 PREGNANT WOMEN, IN RELATION TO TIME OF DELIVERY, ACCORDING TO HEYN⁴

CASE	TIME BEFORE DELIVERY	LEUKOCYTE COUNT	PERCENTAGE OF IMMATURE CELLS AMONG TOTAL NUMBER OF NEUTROPHILIC LEUKOCYTES
1	30 minutes	18,000	27.4
2	2 minutes	18,200	32.4
Average of 20 cases	During labor	15,635	23.0

At each patient's bedside, blood obtained from a finger puncture was smeared on two clean new glass slides. At the same time, blood was obtained for leukocyte counts and the hemoglobin was estimated by the Sahli technic. The time of obtaining the blood samples was recorded in each instance.

The blood counts were made in the usual manner. The smears were allowed to dry in air and were then stained with Wright's stain. A minimum of 300 white blood cells was counted and the cell percentages calculated and recorded according to the Schilling hemogram. The proportion of immature cells to the total number of neutrophilic leukocytes was calculated for each hemogram to furnish an index of the so-called "shift to the left".

FINDINGS

Counts were made in a series of 28 patients before delivery and in a series of 29 patients after delivery. In Table 2, the findings are arranged in order of the interval of time before or after delivery at which blood was taken.

Before Delivery

These patients were arbitrarily divided into two groups: (1) those from whom blood was taken between 0.25 and 9 hours before delivery (average 3.4 hours) and (2) those from whom blood was taken between 10.75 and 237 hours before delivery (average 44.2 hours).

TABLE 2

BLOOD COUNTS ARRANGED ACCORDING TO INTERVAL BEFORE DELIVERY
(28 PATIENTS) OR AFTER DELIVERY (29 PATIENTS)

BEFORE DELIVERY				AFTER DELIVERY			
Time before Delivery		Total Leukocyte Count	Per Cent of Immature Forms among Total Number of Neutrophilic Leukocytes	Time after Delivery		Total Leukocyte Count	Per Cent of Immature Forms among Total Number of Neutrophilic Leukocytes
Hours	Minutes			Hours	Minutes		
	15	18,350	28.8		1 5	24,500	23.1
	47	17,900	33.8		1 10	10,650	38.3
1		9,500	22.2		1 35	18,600	26.5
1		12,950	14.5		2 10	21,700	20.9
1	5	10,350	24.6		2 30	34,400	47.4
1	15	17,500	25.7		2 40	21,650	34.2
1	30	16,750	26.9		2 40	20,050	39.8
1	35	19,750	25.0		2 45	17,350	13.6
2	25	16,950	13.2		2 55	23,000	50.8
2	30	13,400	26.0		3	21,500	30.7
2	40	10,000	19.5		3 15	11,000	23.8
2	40	12,900	12.5		3 25	18,850	33.4
2	50	10,700	26.8		3 43	9,750	12.2
4		13,000	29.2		4 25	15,650	28.9
4	20	9,100	7.8		5	21,450	18.8
4	47	16,550	35.5		5 35	23,850	24.2
7	30	10,850	28.3		5 50	12,450	30.6
7	35	14,600	36.2		6 55	12,700	25.2
8	30	8,750	32.1		8 8	15,200	39.4
9		15,000	30.2		8 15	10,000	46.8
Average count, 20 patients, less than 10 hours before delivery.....					8 20	16,850	28.9
	3	24	13,742	24.9	8 55	10,600	18.7
	10	42	14,250	11.3	9 5	21,300	25.8
	13		11,000	13.8	Average count, 23 patients, less than 10 hours after delivery....		
	13		8,000	8.4	4 30	17,960	29.7
	13	40	13,000	17.7			
	13	50	9,850	17.6	11 40	12,300	12.8
	15	45	12,000	8.8	13	14,550	10.2
	42	35	8,800	12.6	13 19	16,050	20.8
237		7,500	11.1		14 28	8,150	20.8
Average count, 8 patients, more than 10 hours before delivery.....					16	6,800	12.1
	44	54	10,275	12.6	39 10	17,500	32.2
Average count, 28 patients, before de- livery.....					Average count, 6 patients. more than 10 hours after de- livery.....		
	15	12	12,858	21.4	17 54	12,560	18.0
					Average count, 29 patients, after de- livery.....		
					7 12	16,840	27.2

There were 20 patients in the first group and 8 patients in the second group. The shift to the left in the patients from whom blood was taken up to, and including, the nine hour period before delivery was more marked than in those subjects from whom blood was taken more than nine hours before delivery.

The leukocyte count in the first group showed an average of 13,742 cells per cu. mm. (ranging from 8750 to 19,750) with an average shift to the left of 24.9 per cent of neutrophilic leukocytes (ranging from 7.8 per cent to 35.5 per cent). The second group had an average leukocyte count of 10,275 (ranging from 7500 to 14,250) with an average shift to the left of 12.6 per cent (ranging from 8.4 per cent to 17.7 per cent). The average count of the entire series of 28 patients was 12,858 per cu. mm., and the average shift to the left was 21.4 per cent. The average time of taking the smear before delivery was 15.2 hours.

There appeared to be a sudden increase in the "shift to the left" about ten hours before delivery. Prior to this ten hour period, the shift had been only slightly above the so-called normal shift of ten per cent.

After Delivery

These patients were arbitrarily divided into two groups: (1) those from whom blood was taken between 1 and 9.1 hours after delivery (average 4.5 hours), and (2) those from whom blood was taken between 11.6 and 39.1 hours after delivery (average 17.9 hours).

There were 23 patients in the first group and 6 patients in the second group. Study of Table 2 indicates that in the period up to 9.1 hours following delivery, the shift to the left was greater than that occurring in the period between 11.6 and 39.1 hours following delivery.

The leukocyte count in the first group showed an average of 17,960 (ranging from 9750 to 34,400) with an average shift to the left of 29.7 per cent (ranging from 12.2 per cent to 50.9 per cent). The average count in the second group was 12,560 white blood cells (ranging from 6800 to 21,300) and the average shift to the left was 18.0 per cent (ranging from 10.1 per cent to 32.3 per cent). In the entire series of 29 patients, the average leukocyte count was 16,840 with an average shift to the left of 27.2 per cent of total neutrophilic leukocytes. The average time of obtaining the smear after delivery was 7.2 hours.

SUMMARY

Schilling hemograms and total white blood cell counts were made on 57 normal white pregnant women; 28 prior to delivery and 29 following delivery.

The degree of "shift to the left" was indicated by determining the percentage of immature forms among the total number of neutrophilic leukocytes.

In bloods obtained from patients more than ten hours before delivery, the average shift to the left was 12.6 per cent and the average leukocyte count was 10,275 white blood cells per cu. mm. In patients whose blood was obtained less than ten hours before delivery, the average shift to the left was 24.9 per cent and the average leukocyte count was 13,742 white blood cells per cu. mm. In patients whose blood was obtained less than ten hours after delivery, the average

shift to the left was 29.7 per cent and the average leukocyte count was 17,960 per cu. mm. Thereafter there was a tendency for the count to shift to the right.

These studies seem to indicate that the "shift to the left" and the total leukocyte count increase remarkably about ten hours before delivery, continue for approximately ten hours after delivery and then gradually subside.

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CLINICOPATHOLOGIC CONFERENCE*

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Clinical data. During the last part of her pregnancy, a 36 year old white female gained 32 pounds in weight and her blood pressure rose to 185 mm. systolic and 110 diastolic. There was edema of the face and lower extremities and albuminuria. After five days of bed rest, milk diet and mild sedation, the patient's blood pressure decreased to 145 mm. systolic and 80 mm. diastolic. The edema subsided somewhat and there was improvement in her general condition.

Two weeks prior to the expected term of pregnancy, the patient fainted. Soon afterwards, she noticed a bloody vaginal discharge and absence of fetal movements. Upon admission to the hospital on the following morning, physical examination revealed a blood pressure of 160 over 100 mm. and moderate edema of the lower extremities. No fetal heart tones could be heard. The cervix was firm and thick and blood was oozing from the cervical os.

The patient complained of constant pain in the back, lower abdomen and legs. She rapidly became weaker and her pulse became thready. The clinical diagnosis was premature separation of the placenta complicating preeclampsia. It was decided to give her a transfusion of 500 cc. of plasma and to deliver her by cesarian section. The patient was Rh-positive. At operation, the uterus was dark greenish brown, the placenta was completely detached and black blood clots were found at the utero-placental site. Through a low transverse incision, a female stillborn infant was delivered and the old blood clots were removed.

Following a transfusion of 500 cc. of whole blood, the patient's condition improved slightly and the blood pressure rose to 210 mm. over 160 mm. Five hours after operation, a profuse uterine hemorrhage occurred and a uterine pack was inserted. Ergotrate given intravenously had little effect and the uterus did not contract. During the following day, the edema increased gradually and the blood pressure varied between 200 and 150 mm. systolic. During nine days following operation, the patient voided a total of only 600 cc. of urine. To combat shock, 2500 cc. of whole blood was given and, in an effort to establish renal function, ammonium chloride was administered. The general condition of the patient, however, gradually grew worse. On the eighth and ninth postoperative days, the patient had as many as five convulsions a day with twitching of the extremities in the intervals between convulsions. During the last three days before death, the patient talked incoherently and was conscious only for brief periods. On the ninth day after operation, death occurred in uremic coma.

Laboratory data. The urine obtained daily by catheter showed albumin (2 or 3 plus), red and white blood cells and a few hyaline and granular casts. The erythrocyte count after operation varied between 2.5 and 3 million and the leucocyte count between 16,500 and 18,000, with 80 per cent granulocytes. On the

* Received for publication, March 4, 1947.

third postoperative day, chemical examination of the blood revealed 111 mg. of nonprotein nitrogen. The highest value of nonprotein nitrogen was 162 mg. Blood uric acid varied between 7 and 8 mg., while creatinine values were 7.3 mg. on the third postoperative day and 9.6 mg. on the last day.

CLINICAL DISCUSSION

Dr. P. S. Andreson. "The real problem in this case is to determine the nature of the renal failure, which became manifest after the traumatic separation of the placenta, and terminated in uremic death ten days later. While the patient was preeclamptic, her death was certainly not due to eclampsia. In eclampsia, the nonprotein nitrogen of the blood, especially creatinine, never reaches such high values as in this patient. It also seems unlikely that the multiple blood transfusions had anything to do with the fatal outcome. No transfusion reactions were observed; there was no hemoglobinemia and the patient was Rh-positive, which excludes isoagglutination by Rh antibodies. Infection can be ruled out as an etiologic factor in this obstetric complication, because neither local nor general symptoms of a puerperal infection were present.

"The clinical picture of fulminating renal insufficiency reminds one of a condition which McKelvey and MacMahon³ described in 1935. They observed renal failure during or immediately following pregnancy with fatal outcome in a few days. At autopsy, all their cases presented renal lesions characteristic of malignant nephrosclerosis. McKelvey and MacMahon pointed out that in all their cases hypertension existed several years before the last pregnancy. Our patient, on the other hand, had a normal blood pressure during the greater part of her pregnancy. Therefore, it is unlikely that malignant hypertension was an underlying disease in this patient.

"During the bombing of London, British pathologists described renal failure occurring after crushing injuries. They called this the anuric crush syndrome. Autopsy studies of the kidneys of these victims revealed necrosis of the tubular epithelium with blood pigment casts in the lumens of the lower nephrons. In 1942, James Young⁴ reported similar urinary suppression seen in two types of obstetric conditions, retroplacental hemorrhage and trauma of labor. The syndrome included: (1) initial tissue damage, (2) shock, (3) urinary suppression leading to oliguria or anuria and (4) a rise in blood urea which reached its height between the fifth and ninth day.

"From the etiologic and clinical standpoint, I am very much in favor of a diagnosis of anuric crush syndrome, since our case seems to present the four criteria given by Young."

PATHOLOGIC REPORT

Autopsy findings. The weight of the liver was 2250 gm. Its surface had a mottled brownish gray color. Neither gross nor microscopic evidence of focal hemorrhagic necrosis was present. The kidneys were enlarged and weighed 250 and 320 gm. (total 570 gm.). The capsule of each stripped easily and exposed a smooth surface. Dry, yellow, putty-like, triangular areas were found throughout

the cortex and alternated with normal appearing tissue. These yellow infarcts, measuring between 1 and 4 mm., were bordered by serrated, dark hemorrhagic zones and often extended to the capsule. The medullary tissue appeared normal. No gross evidence of occlusion was found in the renal arteries. The uterus was not involuted and measured 10 by 12 cm. In the lower segment, there was a transverse sutured wound measuring 4.5 cm. in length. The uterine wall was soft and its internal ragged surface was covered with dark blood clots. There was no thrombosis of the uterine or pelvic vessels.

Microscopic examination of the kidneys showed triangular necrotic areas with narrow peripheral zones of hemorrhagic infarction. In the necrotic areas, the glomeruli and tubules were completely necrotic. Only a few cells of the interstitial tissue retained the nuclear stain. In the hemorrhagic areas, the glomeruli were engorged and the intralobular arteries and veins were markedly dilated. There was diffuse hemorrhage in the interstitial tissue. In the normal tissue between the infarcts, the tubules were dilated and the epithelium was low, but normally stained. The most striking alterations were evident in the small arteries of the necrotic foci. Their walls were swollen and succulent. The cells composing them were smudgy or disintegrated, and separated one from another by eosinophilic material. In many arteries the individual cells were fused into a bright red mass containing fibrin.

The vascular damage involved the intralobular arteries, but extended into the afferent arterioles and sometimes into the hilus of a glomerular tuft. Some vessels were filled with masses of packed red blood cells without fibrin formation; others contained amorphous hyaline material. The medulla showed moderate congestion of blood vessels but no evidence of tissue necrosis.

CLINICOPATHOLOGIC CORRELATION

Dr. C. A. Hellwig. "Dr. Andreson correctly ruled out eclampsia as the cause of this fatal obstetric complication. The liver did not present focal hemorrhagic necrosis and the kidneys were without glomerular changes typical of eclampsia. There was no suggestion, from the autopsy findings, that bacterial infection of the uterus or transfusion reaction played a rôle in the disease process.

"While the etiologic and clinical features of our case point toward Dr. Andreson's diagnosis of anuric crush syndrome, the autopsy findings leave no doubt that there was no primary tubular necrosis. The primary event in our case was necrosis of the intralobular arteries, with or without thrombosis. This led to an ischemic necrosis of the renal parenchyma supplied by these arteries. Vascular occlusion must have become effective almost simultaneously in all parts of the cortex in both kidneys because the areas of necrosis all appeared to be about the same age.

"Bilateral cortical necrosis is a rare disease and is not invariably associated with pregnancy or preeclampsia, as suggested by Bell,¹ who described this condition under toxemias of pregnancy. Of the 71 cases collected from the literature by Duff and McCre,² 23 occurred in males or nonpregnant females. In the remainder of this group, consisting of 48 pregnant women, cesarian section was performed in



VETRIC SYSTEM 2.

3.

4.

FIG. 1. Cut surface of kidney. Multiple cortical necrotic infarcts with hemorrhagic borders.

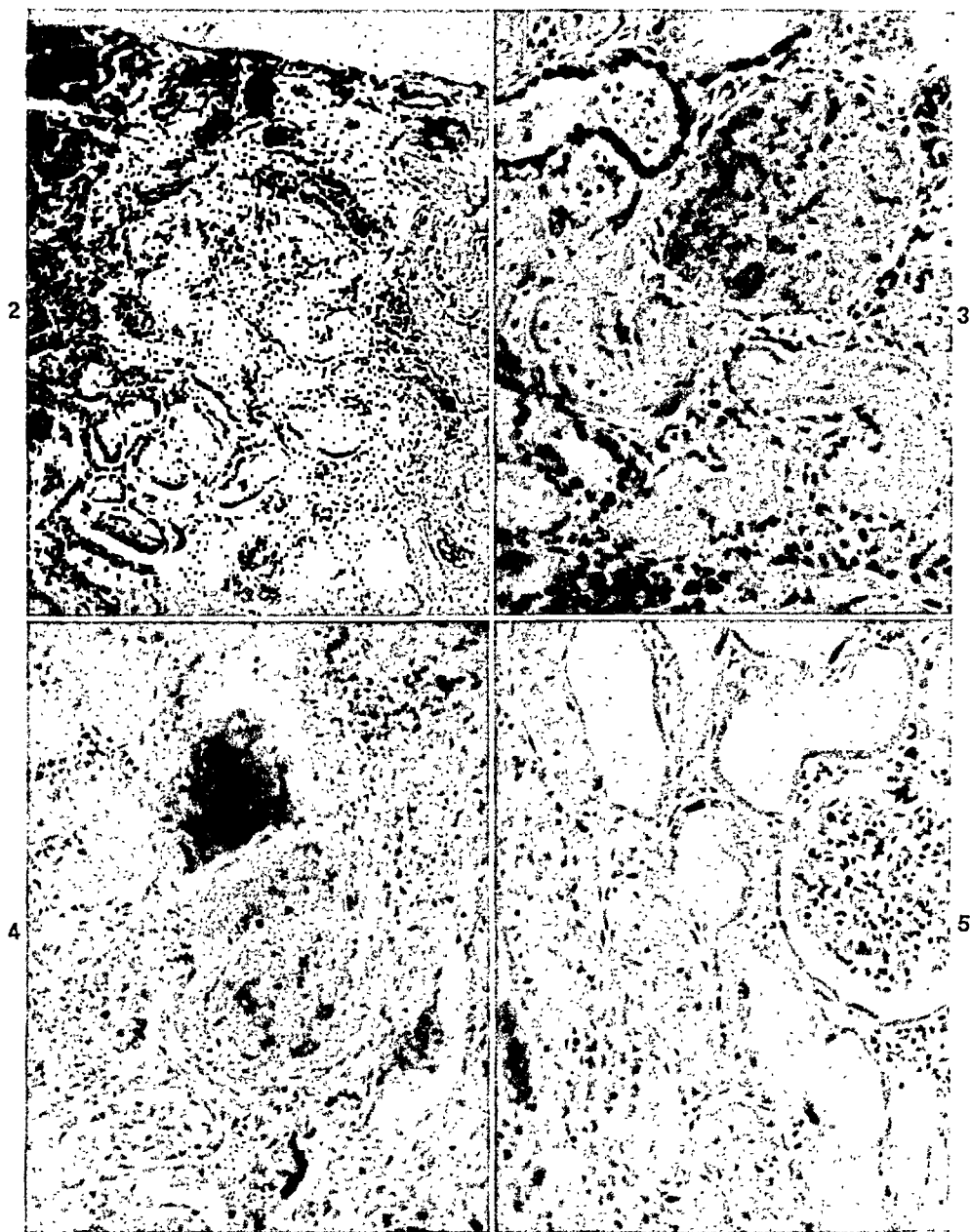


FIG. 2. Microscopic picture of cortex. The infarct does not reach the surface.

FIG. 3. Necrotic glomerulus and tubules. Collecting tube in upper left portion of field is well preserved.

FIG. 4. Thrombocytosis of intralobular artery.

FIG. 5. Cortical area between infarcts.

4 cases, premature separation of the placenta occurred in 21 cases and, in 8 others, preeclamptic symptoms were noted. Among these 48 pregnant women, convulsions were reported in 12 instances before the beginning of anuria, while headaches and disturbances of vision, or both, were mentioned in one-half of the patients.

"The etiology of bilateral cortical necrosis is unknown. Most authors consider that a toxic substance is the underlying cause. It is not known whether this hypothetical agent is exogenous or endogenous, or whether it acts directly on the arterial walls of the renal arteries or by causing intense nervous stimulation.

"The rapid onset and fulminating course of renal cortical necrosis, as well as the anatomic picture of ischemic and hemorrhagic necrosis, has much in common with Schwartzman's phenomenon. The sensitization of the arterial walls of the renal cortex and the provocation of a severe spasm of these vessels could well explain the sequence of events in our case."

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EDITORIAL

THE COMPLEXITIES OF THE RH PROBLEM:

SOME SUGGESTIONS FOR CLARIFICATION

The importance of the Rh problem to the average clinical pathologist is of such magnitude as to demand an understanding of its many complexities. The new knowledge in this field is significant not only to other branches of medicine dealing with transfusion or child birth, but also to the fields of immunology, hematology, genetics and physicochemistry. It is unfortunate that, for a second time, since blood types were discovered in 1900, the nomenclature of blood factors has been confused by multiple systems of terminology. Moreover, the study of the Rh antibodies has entailed the presentation of data which have not been completely compatible with previously conceived theories of the nature of antigen-antibody reactions.

Numerous ingenious technics differing from the classic methods have been evolved for the detection of isoimmunization. As a result, the different specificities of antibodies found in serums of isoimmunized persons, since the original report of Levine and Stetson,¹⁵ have been scarcely simple to follow or easy to comprehend. The naming of this human antigen as the Rh factor because of its resemblance to a similar, but not identical one, discovered in the rhesus monkey by Landsteiner and Wiener⁹ was, in itself, a confusing element.

The accumulation of data since 1939 has developed the problem to such a reasonable degree of maturity as to allow an appraisal of the knowledge and theoretic aspects at this time. To be sure, certain facets are still rough, but an evaluation of the practical nature of postulated theories and explanations, workable for the average clinical pathologist, appears necessary.

The human antigen, now called Rh, was first reported but not named in 1939.¹⁵ A "dominant property" in the fetus inherited from the father and responsible for isoimmunization of the mother was postulated. The specificity of this serum determined on 104 bloods was 80 per cent. Later, it was realized that the human antigen determined by the anti-rhesus serum of Landsteiner and Wiener and called the Rh factor was identical with that responsible for the isoimmunization in the case reported by Levine and Stetson. The conclusion that this agglutinin was inherited as a simple Mendelian dominant was supported by the family studies made by Landsteiner and Wiener.¹⁰ It should be noted in passing, however, that had Levine's anti-Hr¹⁴ serum been discovered first, a similar theory of inheritance of a dominant factor (Hr) could have been proposed equally well. Such a theory, however, would have required diametrically opposed conclusions with the recessive character now becoming dominant and the previously dominant character becoming recessive. The subsequent discovery of many Rh antibodies of different specificities has strongly suggested that all Rh antigens may be determined directly by the use of the appropriate antiserum. Accordingly, in the light of present knowledge, it seems best to abandon all concepts of dominance of Rh genes, whether relative or ab-

solute, and simply consider all Rh types as determined by allelomorphic genes without dominance.

The understanding of the Rh problem, to those who attempted to follow it, seemed beset with difficulties from the very start. For example, it was only shortly after the antibodies having different specificities were first recognized and named that new data required a complete change in their designation. In the naming of Rh genes and antigens and in the formation of subtype classifications, new designations appeared at frequent intervals with the purpose of explaining new data or in an effort to simplify and clarify the known facts. That these efforts were not entirely successful can be seen from examination of Table 1, particularly if the reader has attempted to solve clinical and laboratory problems encountered in cases of isoimmunization with the aid of such designations and classifications.

In Table 1, the classification of Murray¹⁷ represents an ingenious attempt to

TABLE 1
CLASSIFICATIONS OF RH TYPES

WIENER AND LAND- STEINER 1943	WIENER				MURRAY 1944	SYNDER 1945
	1943	1944	Aug. 1946	Nov. 1946		
Rh negative	Rh. neg (W)	rh	rh	rh	rh ₄₅₆	r
Rh ₁	Rh ₁ (U)	Rh ₀ '	Rh ₀ '	Rh ₁	Rh ₁₂₅	R ^{0'}
Rh ₂	Rh ₂ (V)	Rh ₀ "	Rh ₀ "	Rh ₂	Rh ₂₃₄	R ^{0''}
	Rh ₁ , Rh ₂ (UV)	Rh ₀ 'Rh ₀ "	Rh ₀ 'Rh ₀ "	Rh ₁ Rh ₂	Rh ₁₂₃	R ^{0'} R ^{0''}
	Rh (W)	Rh ₀ *	rh ₀	Rh ₀	Rh ₃₄₅	R ⁰
Rh'	Rh' (U)	Rh'	Rh'	rh'	Rh ₁₅₆	R'
	Rh" (V)	Rh"	Rh"	rh"	Rh ₂₄₆	R''
	Rh'Rh" (UV)	Rh' Rh"	Rh' Rh"	rh' rh"	Rh ₁₂₆	R' R''

* Adopted from Race and Taylor.

designate all Rh-Hr antigens in a purely descriptive manner by noting their reactions to the different Rh antisera as required by Fisher's theory. Snyder's²² classification, on the other hand, appears to be a simplification in the method of designation according to Wiener's classification to conform with usual genetic practice. With the exception of the brief and incomplete initial classification of Wiener and Landsteiner,²⁵ the remaining classifications are those of Wiener.^{24, 25, 27, 28} These classifications indicate a remarkable effort to systematize the known facts of the reactions of the Rh antigens into schemes comparable to the four blood groups of Landsteiner. Accordingly, the four groups were designated W, U, V, UV, as determined by two serums (anti-Rh' and anti-Rh"). Further characterization into eight subgroups became apparent through the use of an additional serum (anti-Rh₀). In the three latest classifications of Wiener, these same eight subtypes are continued with slight changes in the symbols designating the individual types. Furthermore, information obtained from extensive family studies was utilized in the integration of gene designation and the theory

of inheritance. This theory postulated inheritance according to Mendelian dominance of six allelic genes, presumably occurring at one locus in each chromosome. One gene was considered dominant to another.²⁶ For example, the gene Rh_1 was dominant over Rh_2 and rh , and Rh_2 was dominant over rh .

One of the most confusing difficulties encountered by many who attempted to use or teach this theory and classification was its failure to explain the significance of the antigen determined by Levine's anti-Hr serum. Levine¹¹ designated this antiserum Hr because of an apparent reciprocal or antithetical relationship between the antigen which it identified and the then designated Rh antigen. This relationship was not clear until Levine recognized that the Hr and Rh' antigens seemed to be related in the same manner that the M and N blood antigens were related.^{12, 13}

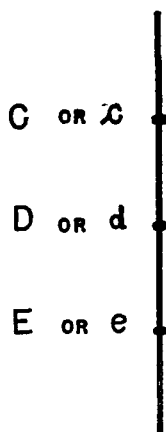


FIG. 1. CHROMOSOME MAP SHOWING POSSIBLE LOCATION OF CDE-cde GENES

cde/cde (Rh-)	CDe/CDe (Rh ₁ Rh ₁)	CDe/cde (Rh ₁ rh)	CDe/cDE (Rh ₁ Rh ₂)	Cde/cDE (Rh ¹ Rh ₂)
14.4%	17.2%	33.0%	11.2%	2.0%
3 antigens	3 antigens	5 antigens	5 antigens	6 antigens

In England, Race and Taylor²⁰ had noted that their St serum (subsequently designated anti-c) could be used to determine whether an individual was homozygous (RhRh of that time) or heterozygous (Rhrh) by negative or positive reactions, respectively. This serum has been shown to be identical to Levine's anti-Hr, although there was an apparent initial discrepancy in specificity as compared to Levine's original serum. Later when the serum with a 70 per cent specificity (now termed anti-C or anti-Rh') was used, the antithetical relationship of Rh' and Hr shown by Levine was discovered independently by Race, Taylor, Cappell and McFarlane.²¹ On this basis, Fisher⁴ assumed that these two antigens were determined by two allelomorphous genes at one locus (*i.e.*, either gene present at this point in one chromosome). But, a lack of a similar relationship of the other Rh antigens to each other or to the then known Hr antigen indicated the possibility of two additional loci on the same chromosome. The arrangement proposed by Fisher⁵ is shown in Fig. 1. Three points on the chromosome, named C, D and E, represent the separate but closely placed locations of genes determining the Rh

antigens. The alternative (allelomorphic) gene possible at each locus is designated c, d or e (determining the three Hr antigens). Thus, the place of the original Hr factor (Hr' or c), as well as the more recently discovered anti-d (Hr₀)² and anti-e (Hr'')¹⁶ become completely clarified. As a matter of fact, this theory predicted the existence of these additional antigens and antisera and thus made their discovery easier.

Figure 2 shows some typical chromosome pairs with the arrangement of the genes determining the CDE (Rh) antigens. It will be seen that the number of antigens may vary from a minimum of three, when homozygous at all three loci, to a maximum of six, when heterozygous at all loci.

Examination of possible arrangements of genes in chromosome pairs according to this theory quickly reveals fascinating possibilities for the clinical pathologist in the study of specific problems. However, in order to estimate the actual CDE gene arrangements (genotype) in a patient without making family genetic studies, it is necessary to have an idea of the incidence of a few of the most common CDE chromosomes. For example, a patient whose erythrocytes contain the C, D and

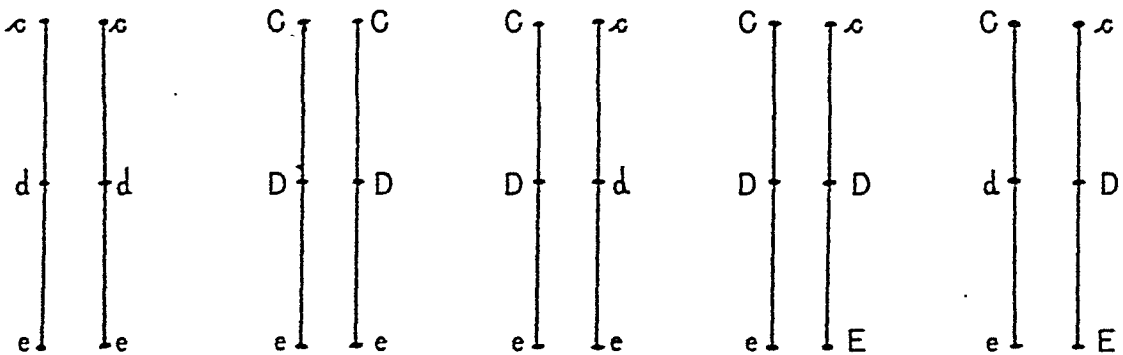


FIG. 2. TYPICAL CHROMOSOME PAIRS DETERMINING RH ANTIGENS (GENOTYPES)

E antigens as determined by the corresponding three antisera, probably has the chromosomes CDe/cDE because the incidence of the chromosome CDE (R₂) is only .13 per cent compared with an incidence for CDe of 43.6 per cent and for cDE of 12.8 per cent. Even with six antisera C, D, E, c, d, e it would still be necessary to use chromosome frequencies to estimate genotypes since each individual has a total of six genes on two chromosomes. Fortunately, the odds for a correct assumption are very good when this method is used in connection with the four antisera C, D, E and c now available for clinical use. Table 2 shows the possible chromosome arrangements under the Fisher-Race theory and their incidence. From this table it may be noted that, in practice, it is only necessary to know that the three chromosomes listed to the left of the double line are the common ones. In any usual situation where alternative chromosomes are possible to explain the antigens determined, the odds are better than 12 to 1 in favor of the three common chromosomes over the rarer ones.

Bearing in mind the well known immunologic principle that an individual can be immunized only against antigens he does not normally possess, it is perfectly simple to visualize any CDE-cde (Rh-Hr) incompatibility, or isoimmunization

problem due to pregnancy. For example, a patient was recently admitted to Baylor Hospital for treatment of a hemolytic transfusion reaction caused by administration of 380 cc. of husband's blood for hemorrhagic shock following delivery of a slightly icteric infant. Owing to continued hemorrhage as well as the hemolytic reaction, the patient was in desperate need of compatible blood. Both husband and wife had been typed Rh-positive elsewhere. A retyping with anti-C, D, E, c and e serums showed the following:

	ANTISERUM					ANTICIPATED GENOTYPE
	C	c	D	E	e	
Mrs. A.....	—	+	—	—	+	cde/cde
Mr. A.....	+	+	+	+	+	CDe/cDE
Infant A.....	—	+	+	+	+	cde/cDE

The genotype of the mother was obviously cde/cde (Rh-negative), while the husband's genotype, anticipated to be CDe/cDE, was shown to be correct when the infant's blood examined later was found to be cDE/cde. From this, it is apparent that the mother could have been immunized against D or E, or both, by the infant and by C, D or E from the husband's blood. The antibodies present were anti-D with development later of anti-C as a result of the

TABLE 2

FREQUENCY OF GENE ARRANGEMENTS ON CHROMOSOMES AND INCIDENCE (ACCORDING TO FISHER'S THEORY)

PER CENT POSITIVE WHITE BLOODS (SPECIFIC- ITY)	ANTIBODY DESIGNATION	COMMON			RARE				
		CDe 43.6 per cent	cde 37.9 per cent	cDE 12.8 per cent	cDe 3.0 per cent	cdE 1.7 per cent	Cde 0.8 per cent	CDE*	CdE**
70	Anti-C	+	—	—	—	—	+	+	(+)
85	Anti-D	+	—	+	+	—	—	+	(—)
30	Anti-E	—	—	+	—	+	—	+	(+)
80	Anti-c	—	+	+	+	+	—	—	(—)
65	Anti-d	—	+	—	—	+	+	—	(+)
96	Anti-e	+	+	—	+	—	+	—	(—)

* Calculated by Race, 0.13 per cent.

** Predicted.

transfusion. The infant's red cells contained only D and E antigens to the three standard serums and were strongly positive to anti-c serum. Note that the husband's genotype, CDe/cDE, having genes CD on one chromosome and DE on the other has the clinical significance that any future pregnancy would result in an erythroblastotic infant.

In another case to be reported in detail elsewhere,⁸ we were presented with the problem of supplying blood for an urgently needed transfusion in a pregnant woman in another state who had had a hemolytic reaction to transfused blood

from a donor, not her husband. One child was living and one premature infant had died. Several transfusions had been given prior to the reaction. The mother was type A, with anticipated genotype CDe/CDe by use of anti-C, D, E and c serums. The father was expected to be CDe/cde and the living child CDe/cde. The patient had received transfusions of blood of probable genotypes CDe/cDE, cDE/cDE, cDE/cde in the order listed. Note that the rare anti-e serum of Mourant was not actually necessary in the management of this case, although it was essential to complete the genotyping of the second (cDE/cDE) and third (cDE/cde) bloods of the series given. An antibody demonstrable only with the developing test or Diamond's albumin method was too weak for accurate determination of specificity but appeared to be anti-c or d. From the estimated genotypes, it is perfectly obvious that the patient might have been immunized by c, d or E. For this reason it was decided to give only blood negative for these antigens, that is, identical to that of the patient. In choosing such bloods of identical genotype it was necessary to use the method of predicting probable

TABLE 3
ESTIMATED GENOTYPES
(Rare types omitted)

	HETEROZYGOTE	HETEROZYGOTE
Determined by anti-C, D and E	Determined by anti-c	Determined by anti-e
cde/cde		
Cde/Cde	Cde/cde	
cdE/cdE		cdE/cde
cDe/cde		
CDe/CDe	CDe/cde	
cDE/cDE		cDE/cde
CDe/cDE		

genotypes, using only the four serums anti-C, D, E and c, since anti-d was not available. No difficulty resulted from giving four pints of blood selected in this manner. The use of the Fisher-Race concept and the method of estimating genotypes greatly facilitated the management of this case.

Table 3 includes an arrangement of the more common genotypes encountered by the clinical pathologist in practice. Again it may be noted that all major genotypes may be estimated by use of anti-C, D, E and c serums presently available for clinical use. A possible exception of some importance in obstetric practice is the instance where differentiation between the homozygous cDE/cDE and heterozygous cDE/cde is desired. For this purpose the rare anti-e serum would be required.

For practical application by clinical pathologists, it seems that the use of the Fisher-Race concept and nomenclature can be of the greatest value. The adoption of this nomenclature and method of approach formulated as the result of the brilliant cooperative research of British workers in no way reflects on the earlier original discoveries of American investigators, but again emphasizes that science

is international. While it may well take some period of time for the accumulation of sufficient evidence to satisfy geneticists as to the ultimate correctness of the different theories of inheritance and systems of nomenclature, the clinical pathologist is daily confronted with problems which he can solve more easily by the concept of Fisher and Race. In practical application with a large transfusion service, in teaching medical students and technicians, and even in explaining incompatibility problems of erythroblastosis or of transfusions to patients, we have found the CDE system to be most satisfactory. This experience includes the use of anti-C, D, E, C^w, D^u, c and e serums.* Diamond,² after extensive experience with similar antisera including his anti-d has found the Fisher-Race nomenclature most useful. Chown¹ and Waller²³ have employed this nomenclature to advantage in recent publications.

The adaptability of the Fisher-Race concept is well illustrated, first, by making possible the prediction of types of antigen combinations and antisera^{2, 6, 16, 21} not previously observed and, second, by the incorporation of the unpredicted additional rare allelomorphs C^w and D^u at the C and D loci. It is perfectly possible that rare additional allelomorphs may be found at the E locus also. At present, the clinical pathologist need not concern himself with these recently discovered antigens because of their rarity and the scarcity of the antisera. A further observation based on statistical studies is Fisher's suggestion that the order of the genes on the chromosome may be D, C and E.^{18, 19} The order of genes in the chromosome makes no difference whatsoever for clinical use but it is of interest genetically.

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BOOK REVIEWS

Disorders of the Blood. Ed. 5. By SIR LIONEL E. H. WHITBY, M.D. (Cantab.), F. R. C. P. (Lond.), Regius Professor of Physic in the University of Cambridge, Honorary Consulting Haematologist to the Army; and C. J. C. BRITTON, M.D. (New Zealand), D.P.H., Assistant Pathologist, the Bland-Sutton Institute of Pathology, The Middlesex Hospital, Pathologist, The Samaritan Free Hospital for Women. 665 pp., 15 plates (10 colored), 71 figs. \$10.00. Philadelphia: The Bakiston Company, 1946.

This authoritative text on hematology has been extensively rewritten and contains much new material, bearing particularly on the origin and development of the blood cells, hemolytic anemias, blood transfusions and hemagglutination, and the theoretical and clinical implications of Rhesus factor inheritance and immunization.

The authors' intimate familiarity with the world's hematologic literature has led to a comprehensive yet critical coverage of the subject matter. Moreover, the importance of the book as a reference text is equaled by its clarity and readability. Such confusing and controversial topics as nomenclature and theories of blood cell formation are dealt with in an understandable and logical manner. The emphasis upon the dynamic nature of cell development permits an interpretation of pathologic states in terms of abnormal physiology.

One of the chief merits of this work lies in its bridging of the gap between the points of view of the internist and the clinical pathologist. Diagnostic methods and pathologic variations in the blood and hematopoietic tissues are described with thoroughness and accuracy, and clinical problems are discussed with the authority of wide experience in their management.

Ann Arbor, Michigan

FRANK H. BETHELL

Hygiene. Ed. 4. By FLORENCE L. MEREDITH, M. D., Professor of Hygiene and Public Health, Tufts College. 838 pp., 155 figs. \$4.00. Philadelphia: The Blakiston Company, 1946.

This admirable text for college students now appears in a fourth edition with a number of changes from the third edition published five years ago. The author has sought in this edition, as previously, to present a picture of health as it should be interpreted for the individual and, at the same time, to indicate the rôle of the community in public health.

An over-all picture of the health of the people of the United States is given by way of introduction. A brief introduction to anatomy and physiology, with a simple description of disease processes and the mechanism of recovery, prepares the student for the chapters in Part III on Forces for Health which are available through medical science and the physician, and through organized community effort in the field of public health.

Parts IV and V present a number of the more important health problems of the individual and, at the same time, emphasize how these problems are related to the public health.

Part VII on Hygiene of Everyday Life deals very completely with bodily functions, the effect of environmental factors, and the proper understanding of these so that good health may be maintained. As evidence that the text is up-to-date, there is a simple and understandable discussion of the effects of altitude in flying.

The hygiene of sex and reproduction and the importance of mental health are dealt with clearly and conservatively.

Altogether, the author has prepared a very useful book for college students and other nonmedical persons. Such readers should profit greatly through the study of this work, not only as individuals, but also as parents seeking better understanding of the health of the family, and as citizens seeking the best in health for the community.

Detroit

BRUCE H. DOUGLAS

The Compleat Pediatrician. Practical, Diagnostic, Therapeutic and Preventive Pediatrics. Ed. 5. By W. C. DAVISON, Professor of Pediatrics, Duke University School of Medicine. 253 pp., 29 tables. \$4.00. Durham, North Carolina: Duke University Press, 1946.

In the fifth edition, this popular book has remained unchanged in size and shape, but much of the material has been rewritten and many additions reflect the latest developments in the field of pediatrics. As in the earlier editions, an enormous body of data is condensed into a minimum of space. In spite of the excellence of the clinical descriptions and the usefulness of the numerous tables, the result is not entirely satisfactory. Perhaps the most valuable chapters are those which reflect the author's large practical experience in handling sick and healthy children, especially the chapters on Feeding, General Therapy and Pediatric Nursing and Growth, Development and Child Care. Elsewhere, the author's aim to be both concise and complete has forced him to present many observations without the necessary qualifying and limiting statements; the resulting bald residual sentences which make up the text tend to give a distorted impression of facts. Written for the practitioner, who has no ready means for checking or critically interpreting such statements, and who is, therefore, likely to accept all statements literally, the book is not without danger. This is all the more evident when controversial issues are handled in the same abbreviated dogmatic manner as are established facts. To give only one example, in the paragraph on erythroblastosis fetalis, these statements are made: "Rh tests for marriage are as important as those for syphilis. If the wife is Rh- & the husband Rh + or suspected because of previous miscarriages, birth control is advisable. If the wife becomes pregnant, her anti-Rh titer should be frequently tested and if it reaches a dangerous level, Caesarean section is recommended followed by 'safe' transfusions." Such recommendations considerably overstep the authority of a book written "to serve as a ready reminder" for the physician.

In view of the author's avowed purpose to write a practical book, it is surprising to find the text burdened with items which can be of little practical value and might almost be called oddities, such as information on the serum cholesterol and complement titer in rheumatic fever and the unqualified statement that "the tourniquet, formol-gel & Weltmann reactions are positive; the last is the most helpful."

An entire chapter is devoted to laboratory procedures, many of them far too ambitious for office use. Since this part will hardly be used by the experienced laboratory worker, one feels that the book would benefit if this chapter were deleted or materially shortened and the space thus saved were used for amplification of the clinical chapters.

Detroit

W. W. ZUELZER

Blood Grouping. By WILLIAM C. BOYD, J. W. CAMERON, L. K. DIAMOND, PHILIP LEVINE, M. MELIN, J. L. ONCLEY, LOUIS PILLEMER, D. A. RICHERT, EVE B. SONN, A. S. WIENER, and ERNEST WITEBSKY. 133 pp., 11 figs., 44 tables. New York: Annals of the New York Academy of Sciences, 1946.

A conference on Blood Grouping was held by the Sections of Biology, and Physics and Chemistry of the New York Academy of Sciences on May 18 and 19, 1945. The published transactions contain six articles preceded by a brief introduction by Boyd. The scope of the first article by Witebsky is described by the title: Isolation and Purification of Blood Group A and B Substances; Their Use in Conditioning Universal Donor Blood, in Neutralizing Anti-Rh Sera, and in the Production of Potent Grouping Sera. Included in the discussion of the paper is an interesting brief communication on the estimation of isoantibodies by the quantitative precipitin reaction by Kabat and Bezer.

The second article by Oncley and associates deals with chemical methods employed in the fractionation and concentration of anti-A, anti-B, and anti-Rh isoagglutinin reagents, with resulting utilization of from five to eight times as many donors as could be effectively used without this procedure and at least eightfold concentration of the reagents. Pillemer's contribution evaluates various factors which influence the titer of isoagglutinins and their avidity (*i.e.*, rapidity and intensity of reaction on the slide).

In his article, Boyd confirms the well known variation in reactivity of cells of different individuals of groups A and AB. Levine discusses genetic and constitutional causes of

fetal and neonatal morbidity in a good general review. In the final paper, Wiener and Sonn review the historic development of Wiener's conception of the nomenclature of the Rh factor. The comparison with the British nomenclature is further emphasized by the discussion of Dr. R. R. Race.

The New York Academy of Sciences has rendered a service, first by sponsoring the Conference, and then, by publishing the transactions. Students of the subject of blood groups in general, and of the Rh factor in particular, will welcome this small but informative volume.

Chicago

I. DAVIDSOHN

Progress in Gynecology. Edited by JOE V. MEIGS, M.D., Clinical Professor of Gynecology, Harvard Medical School; and SOMERS H. STURGIS, M.D., Chief, The Vincent Memorial Hospital Laboratory. 552 pp., 73 figs., 1 color plate, 16 tables. \$7.50. New York: Grune & Stratton, 1946.

This book is a compilation of papers on gynecologic subjects, published as a refresher for medical men who have spent the past few years in the armed forces and, as a result, have been unable to keep abreast of the recent advances in this field.

Approximately 70 authors have contributed to this volume, each writing on a subject of particular interest or familiarity to him. A large portion of the book is devoted to endocrinology; physiology and diagnosis are also treated; and a minimum of space is allotted to pathology and to postoperative technic. Chapters are devoted to growth and physiology, diagnostic methods, functional disorders, interrelationship of endocrine glands, sterility and reproduction, infections and their treatment, benign growths, malignant growths, operative techniques and preoperative and postoperative care.

The editors have purposely included scant bibliographic references, which is unfortunate, since many of the papers are so abbreviated that the interested reader will, of necessity, require additional material.

A number of articles are especially good. Schiller has an excellent paper on the embryology of functional ovarian tumors. The Smiths review their work on menstrual toxin and its relation to the mechanism of menstruation. They offer an ingenious explanation of the rôle of menstruation as a pituitary stimulant. Markee discusses briefly the vascular changes in the uterus in relation to menstruation. Rakoff's paper on, "Vaginal Smears for the Determination of Ovarian Function" is excellent.

The article on the diagnosis of uterine cancer by vaginal smears by Fremont-Smith and Graham, on the other hand, is so brief as to be of little value to the reader who has been out of touch with this new diagnostic method. It would have been greatly improved by a few illustrations.

Gynecologic endocrinology is given a large proportion of space and most of the articles in this field are excellent. Of particular merit is the section on sterility by Rock, Simmons, Walter Williams and Eastman. Te Linde presents an excellent treatise on the relation of "Intra-Epithelial Carcinoma" to invasive cancer of the cervix. The photomicrographs included are noteworthy in this otherwise poorly illustrated book. The section on operative technic is limited. It includes a regrettably brief article by Meigs on his work with the Wertheim-Taussig operation for cervical carcinoma. A valuable feature of the book is an enclosed loose-leaf chart of commercial preparations of endocrine products.

Obviously, this work is not intended to serve as a textbook. It fulfills its stated purpose as well as the limitations of space permit. It would have been desirable to have had a few additional pages in order to permit a more complete discussion of some of the subjects, and to have included more illustrations and references. In general, the book is presented in a popular vein, and clinical pathologists will find many items of interest in its pages.

Detroit

EMIL D. ROTHMAN

Practical Physiological Chemistry. Ed. 12. By PHILIP B. HAWK, Ph.D., President, Food Research Laboratories, Inc.; BERNARD L. OSER, Ph.D., Director, Food Research Laboratories; and WILLIAM H. SUMMERSON, Ph.D., Associate Professor of Biochemistry,

Cornell University Medical College, New York City. 1323 pp., 329 figs., 121 tables, 5 color plates. \$10.00. Philadelphia: The Blakiston Company, 1947.

It is now 40 years since the first edition of *Practical Physiological Chemistry* was published and 10 years since the eleventh edition was written by Hawk and Bergeim. In this latest edition, Hawk has collaborated with two new authors in a complete revision and enlargement of the previous edition. This edition contains an additional 355 pages. Obsolete material has been deleted and much new material added, including sections on the polarograph, isotopes, sulfa drugs, metabolic antagonists and antibiotics, the Warburg tissue-slice procedure, photometric analysis, electrophoretic fractionation of plasma proteins, composition of foods, and vitamins and their clinical relationships.

This work is unusual in that it has so long served as a standard textbook both in the college classroom and the hospital laboratory. The style is clear and direct and the subject matter brief and understandable. The theoretical considerations and experiments are supplemented by practical methods and applications. There are numerous structural formulas which should be valuable. The appendix contains information concerning preparation of reagents and solutions; tables on the nutritive value of selected foods, including content of minerals and vitamins; and data on maintenance of animals for experiments in nutrition. The popularity which the book has enjoyed in successive editions is fully deserved. This volume should be in the library of every biochemical laboratory.

Fundamentals of Clinical Neurology. By H. HOUSTON MERRITT, M.D., Professor of Clinical Neurology, College of Physicians and Surgeons, Columbia University, Chief of the Division of Neuropsychiatry, the Montefiore Hospital; FRED A. METTLER, M.D., Ph.D., Associate Professor of Anatomy, College of Physicians and Surgeons, Columbia University; and TRACY JACKSON PUTNAM, M.D., Professor of Neurology and Neurological Surgery, College of Physicians and Surgeons, Columbia University, N. Y. 289 pp., 96 illus. \$6.00. Philadelphia: The Blakiston Company, 1947.

This is a concise and clearly written text on diseases of the nervous system presented for the practitioner of medicine. Part I is concerned with examination of the nervous system and Part II with anatomic diagnosis. The book is surprisingly easy to read. The titles and subtitles are well chosen. There is good correlation of clinical, anatomic, physiologic and pathologic data. The last chapter of 26 pages is devoted to laboratory findings in the cerebrospinal fluid in various diseases. Findings are listed for 75 "cerebrospinal fluid syndromes". The book is useful and can be recommended without reservation.

NEWS AND NOTICES

MONTANA SOCIETY OF PATHOLOGISTS

On February 15, 1946, all five pathologists of Montana met in Butte and organized the Montana Society of Pathologists. Present were: Dr. Mary Martin, Billings; Dr. Joe Ohlmacher, Missoula; Dr. T. F. Walker, Great Falls; Dr. Eugene Hildebrand, Great Falls; and Dr. R. F. Peterson, Butte. Dr. R. F. Peterson was elected president and Dr. Mary Martin, secretary.

The constitution and by-laws adopted follow the principles established by the American Society of Clinical Pathologists and the Illinois Society of Pathologists. The Society plans to hold two meetings a year. A meeting has already been held with representatives of the State Board of Health, who have asked the pathologists to act as a consulting board for the hygienic laboratory of the state.

REVISED BIOLOGICS REGULATIONS

Dr. M. V. Veldee, Chief, Biologics Control Laboratory, National Institute of Health, writes as follows:

"It is desired to call attention to a revision of the regulations governing the manufacture and sale of biologic products published in the Federal Register on January 21, 1946, and effective 30 days after that date.

"As is indicated in these regulations, the scope of the Biologics Law is broadened somewhat and certain diagnostic products administered or applied to a person or prepared from or with the aid of a biologic product should now be distributed in interstate commerce only if prepared by a licensed laboratory.

"Also requiring a license for distribution are blood grouping and Rh typing serums, for which minimum requirements have been prepared in anticipation of the revision of the regulations.

"Information as to the status of any of these products, or the requirements applicable to them, may be obtained from the Biologics Control Laboratory, National Institute of Health, Bethesda 14, Maryland."

POSTGRADUATE COURSES

The University of California Medical School announces the following courses:

1. *Course in the Applications of Nuclear Physics to the Biological and Medical Sciences.* June 30 to July 18 inclusive, 1947. This course will consist of didactic lectures, laboratory demonstrations and seminars for round table discussions, and will be open to individuals in the fields of medical and biologic research.

2. *Course in Gynecological and Obstetrical Pathology, and in Smear Technique as it Pertains to Cancer of the Uterus, Stomach, Urinary Tract and Lungs.* August 4 to 15 inclusive, 1947. This course is limited to 75 and registrants are to furnish their own microscopes, which should be of good quality.

Requests for detailed information should be addressed to Stacy R. Mettier, M.D., Head of Postgraduate Instruction, Medical Extension, University of California Medical Center, San Francisco 22, California.

THE MUCOPOLYSACCHARIDE CONTENT OF THE SKIN IN LOCALIZED (PRETIBIAL) MYXEDEMA*

E. M. WATSON, M.D., AND R. H. PEARCE, B.S.

From the Department of Pathological Chemistry, Faculty of Medicine, University of Western Ontario, and the Department of Clinical Pathology, Meek Memorial Laboratory, Victoria Hospital, London, Canada

The occurrence of localized myxomatoid cutaneous lesions, involving chiefly the anterior aspects of the legs of persons with present or past hyperthyroidism, has been recognized for many years and identified by a variety of descriptive terms such as "localized solid edema of the extremities in association with exophthalmic goiter",¹² "circumscribed myxedema",¹⁴ "localized myxedema with hyperthyroidism",⁴ "mucoid degeneration of the skin associated with hyperthyroidism",⁷ "localized pretibial myxedema"¹⁶ and "myxedema circumscriptum thyrotoxicum".³ Although comprehensive reviews of the clinical and cytologic features of the abnormality have been given by numerous writers,^{1,3,14,16,17} the biochemical aspects of the disorder have received but scant attention.

The relationship, if any, of this particular pathologic condition to dysfunction of the thyroid gland has not been established. The fact of its development during the active stage of toxic thyroid disease, however, as well as the resemblance of the cutaneous changes to those of generalized myxedema, has been commented on by several observers. The characteristic histologic features consist of the presence of mucin or a muciform substance in the corium, demonstrable by special staining technics, and an associated splitting of the connective tissue fibers. Hyperkeratosis is often present. There are no signs of inflammation or lymphatic stasis.

The mucins, properly termed mucoproteins, are saltlike combinations of carbohydrate and protein. These compounds, because of their lyophilic colloidal nature, produce extremely viscous aqueous dispersions, even in low concentration, from which they are precipitable by acidification with acetic acid. Their physicochemical properties are determined largely by the characteristics of the carbohydrate moiety which contains an amino sugar; hence the apposite expression mucopolysaccharide.

The particular compounds which are of immediate interest contain, within their molecular structure, uronic acid in addition to an aminohexose and usually acetyl and sulfate groups as well. Such substances are referred to as "acid" mucopolysaccharides. In this connection, Levene⁶ showed that chondroitin-sulfuric acid was composed of equimolecular amounts of N-acetyl galactosamine, glucuronic acid and sulfuric acid; while Meyer and Palmer¹⁰ identified a sulfur-free acid mucopolysaccharide, made up of N-acetyl glucosamine and glucuronic acid in equimolar quantities, which they named hyaluronic acid. Meyer and

* Aided by a grant from the Division of Medical Research of the National Research Council of Canada. Received for publication, April 2, 1947.

Chaffee⁸ subsequently isolated hyaluronic acid and a substance resembling chondroitinsulfuric acid from pig skin.

Carol¹ extracted with weak alkali from the affected skin of a thyrotoxic patient with so-called pretibial myxedema a substance which, after precipitation with acetic acid and boiling for one-half hour with 5 per cent hydrochloric acid, developed reducing power with respect to Fehling's solution. Chain and Duthie,² without presenting any evidence, stated that there seemed to be an accumulation of hyaluronic acid in the skin of myxedematous subjects. Trotter and Eden¹⁶ suggested that the material which accumulates in the skin in atypical (pretibial) myxedema may be hyaluronic acid and that the lesions might be due to an abnormal metabolism of this substance.

ANALYTIC DATA

An attempt was made to investigate the chemical nature of the mucinous material which was present in specimens removed for biopsy of the affected skin from the legs of two patients with so-called pretibial myxedema.¹⁷

The specimen from one of the patients (Case 2) had been placed inadvertently in formalin fixative before it was obtained for chemical analysis. The effect of this solution in altering the original weight and the mucopolysaccharide content of the skin is unknown. However, 15 gm. was considered to be the probable initial weight of this specimen.

The tissue was treated in essentially the same manner as that employed by Meyer and Palmer¹⁰ for the isolation of hyaluronic acid from vitreous humor and umbilical cords.

The skin samples, each of which included both the cutis and the epidermis, were cut into small pieces and dried in several successive changes of acetone. Blood pigment and other cellular products were removed by washing the dehydrated tissue with 90 per cent acetic acid, which in turn was removed by further washing with 95 per cent ethanol. The nearly colorless residue was extracted several times with cold water at neutral reaction and the mucoproteins were precipitated from the extract with 6 volumes of alcohol acidulated with acetic acid. This extraction was repeated until no further precipitation occurred with the acid alcohol. The solid substance thus obtained was considered to represent the hyaluronic acid fraction.

The material which remained following the above-described extraction process dissolved almost completely in a 3 per cent solution of sodium hydroxide. By treating this alkaline extract with an excess of barium carbonate on a boiling water bath, concentration and denaturation of the protein were accomplished simultaneously. The centrifugate of this product was precipitated with 10 volumes of glacial acetic acid. The precipitate was considered to be the chondroitinsulfuric acid fraction, since the isolation procedure employed was comparable to that described by Levene⁶ for the preparation of chondroitinsulfuric acid from sclerae.

A portion of the skin from the amputated leg of an elderly man (Control A) was subjected to the same treatment, although it was realized that the ideal source of

the "control" data would have been the unaffected skin of the patients with the localized myxedema.

The dry weights of the substances isolated from the cutaneous samples, relative to the weights of the fresh specimens of skin, are shown in Table 1. No reasonable explanation can be offered for the notable difference in the results in the two patients under consideration other than its possible relationship to the fact that the tissue specimen from Case 2 was exposed to preliminary contact with formalin.

ISOLATION OF THE MUCOPOLYSACCHARIDES

At this point, the investigation was directed toward a more intensive study of the mucopolysaccharides normally present in human skin. The experience gained in the latter undertaking served to emphasize the inadequacy of the methods which had been employed in the preliminary analyses.

The hyaluronic acid fractions of the preparations undoubtedly were contaminated with protein and glycogen since no effort had been made to remove

TABLE 1
THE CRUDE MUCOPROTEIN FRACTIONS FROM THE SKIN OF PATIENTS WITH
PRETIBIAL MYXEDEMA

SOURCE OF MATERIAL ¹⁷	FRESH WEIGHT OF SKIN IN GM.	"HYALURONIC ACID",* IN MG. PER 100 GM. SKIN	"CHONDROITINSULFURIC ACID", † IN MG. PER 100 GM. SKIN
Case 1	3.68	1300	30
Case 2	15.(?)	230	1930
Control A	5.69	40	trace

* Aqueous extract precipitated with alcohol.

† Protein-free alkaline extract precipitated with acetic acid.

these substances. Likewise, the product which had been considered to be chondroitinsulfuric acid probably contained traces of proteins and their derivatives and glycogen, in addition to the mucopolysaccharides which remained after the aqueous extraction.

Unfortunately, the chondroitinsulfuric acid fraction from one patient (Case 2) was the only material remaining in sufficient quantity to permit further investigation. An effort was made, however, to further purify this substance by a process similar to that employed by Meyer and Chaffee⁵ for the isolation of the mucopolysaccharides from pig skin. The residual protein was removed first by shaking a solution of this fraction with a chloroform-amyl alcohol mixture, and centrifuging down the denatured proteins. This step was followed by the adsorption of any remaining protein on Lloyd's reagent from normal acetic acid solution. The protein-free substance was rid of glycogen by incubating with filtered saliva. The purified mucopolysaccharides were then fractionated into hyaluronic acid and chondroitinsulfuric acid portions by treating their barium salts with alcohol.

In Table 2, the yields of these materials are compared with those of similar fractions isolated from control samples of human skin by the method of Meyer and Chaffee.⁸ Control B represents the average of values derived from the analysis of nine different specimens of fresh skin obtained from legs which had been amputated above the knee because of peripheral gangrene or injury. The sex and age of the subjects did not appear to influence the findings appreciably. The relative amounts of the mucopolysaccharides present in Case 2 were calculated on the basis of an assumed weight of 15 gm. for the fresh skin.

TABLE 2
YIELDS OF PURIFIED MUCOPOLYSACCHARIDES FROM "CHONDROITINSULFURIC ACID"
FRACTION OF SKIN FROM PATIENT WITH PRETIBIAL MYXEDEMA

SOURCE OF MATERIAL	MG. MUCOPOLYSACCHARIDE PER 100 GM. FRESH SKIN	
	Hyaluronic acid	Chondroitinsulfuric acid
Case 2 ¹⁷	310	180
Control B	26 ± 9	28 ± 6

TABLE 3
PROPERTIES OF MUCOPOLYSACCHARIDE PREPARATIONS FROM THE AFFECTED SKIN OF A
PATIENT WITH PRETIBIAL MYXEDEMA

SOURCE OF MATERIAL	HYALURONIC ACID		CHONDROITINSULFURIC ACID	
	Apparent hexosamine content, in per cent	Degree of enzymatic hydrolysis, in per cent	Apparent hexosamine content, in per cent	Degree of enzymatic hydrolysis, in per cent
Case 2 ¹⁷	13	44	15	8
Control B	22 ± 8	61 ± 15	20 ± 5	-0.6 ± 2.7
Range	11 to 34	40 to 96	12 to 28	-5.5 to 3.3

Identification of the mucopolysaccharide fractions

Ordinarily, 50 mg. of the pure materials is considered to be the minimum necessary for the unequivocal identification of the acid mucopolysaccharides by the conventional methods. Consequently, the analysis of the limited amount of material which was available in this study yielded data which were, at best, only suggestive of the true nature of the compounds which had been contained in the skin.

A semiquantitative estimation of the hexosamine content was accomplished by a modification of the method of Palmer, Smyth and Meyer.¹³ The extent of the hydrolysis of the preparations by the action of hyaluronidase* was estimated by the release of N-acetyl hexosamine as determined by the method of Morgan

* Kindly supplied by the Schering Corporation, Bloomfield, New Jersey, through the courtesy of Dr. Erwin Schwenk.

and Elson.¹¹ The presence of uronic acid in these hydrolysates was established qualitatively by the naphthoresorcinol test.

The results of these analyses, compared with the results obtained under similar circumstances from the control samples of human skin, are shown in Table 3.

DISCUSSION

By the use of methods which have been employed successfully by other analysts for the separation of the mucopolysaccharides from animal tissues, certain materials were isolated from the affected skin of two patients with so-called pretibial myxedema. The presence of acetylhexosamine and uronic acid in each of the two fractions indicates that the latter belong to the acid mucopolysaccharide group of compounds.

The marked response of one fraction to the action of the enzyme hyaluronidase would seem to identify it as hyaluronic acid. While this enzyme is known to be capable of depolymerizing other acid mucopolysaccharides, no record has been found of a like degree of hydrolysis occurring with any acid mucopolysaccharide other than hyaluronic acid. Indeed, the extent to which the hydrolysis progressed agrees with the results obtained by Meyer *et al.*⁹ under similar conditions.

Beyond associating it with the acid mucopolysaccharides, a definite identification of the second fraction was not attained. However, it is not improbable that it was related to the substance resembling chondroitinsulfuric acid which Meyer and Chaffee⁸ isolated from pig skin. Nevertheless, the possibility of its being mucoitinsulfuric acid or some other unrecognized acid mucopolysaccharide must be conceded.

The data presented here, although inconclusive, add support to the opinion advanced by Trotter and Eden¹⁶ that hyaluronic acid may be involved in the lesions of pretibial myxedema. Indeed, it would appear that the other acid mucopolysaccharides in the skin are increased also. This pronouncement invites speculation concerning the possible explanations for the biochemical disturbance underlying this abnormality, as well as for the peculiar localization of the particular substances in the skin. While various theories pertaining to the pathogenesis of so-called pretibial myxedema have been advanced,³ the nearest approach to a rational explanation would seem to be on the basis of a local disturbance of the metabolism of the mucopolysaccharides.

Trotter and Eden¹⁶ intimated also that hyaluronidase might exert a controlling influence upon the deposition of "mucin" in the skin and they further suggested that the apparent increase of cutaneous "mucin" in myxedema might be due to a deficiency of hyaluronidase. It is not unreasonable to presume that inactivation of the enzyme would accomplish the same end result. The demonstration of hyaluronidase (spreading factor) in the normal thyroid gland may be of pertinent significance¹⁵ and, recently, Haas⁵ has reported the existence in blood of an enzyme (antinvasin) capable of destroying hyaluronidase. If confirmed, many of these suggestions and observations may prove to be of considerable pathologic importance.

SUMMARY

The affected skin of two patients with pretibial myxedema contained a marked excess of acid mucopolysaccharides, including a substance exhibiting the characteristics of hyaluronic acid.

Acknowledgment. The writers wish to express their appreciation of the assistance in this work provided by Dr. H. L. Williams, a former associate.

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PROGRESS IN CANCER RESEARCH*

WALTER M. SIMPSON, M.D.

From the Charles F. Kettering Foundation, Dayton, Ohio

Since October 1945, I have been engaged in surveying the field of cancer research for the Charles F. Kettering Foundation. The survey has concerned itself with: (1) the progress made in cancer research during the war years; (2) what can be done to control cancer with the weapons now available; and (3) the most hopeful leads from present research on the nature of cancer which bear on its cause, prevention and cure.

PROGRESS DURING THE WAR

The urgent requirements of the armed services for research related directly to the war effort caused a serious depletion in the ranks of those who had been engaged in cancer research in this country prior to Pearl Harbor. Nevertheless, considerable work was carried on during the war years, particularly in the biologic, chemical and physical aspects of the cancer problem, in such institutions as the National Cancer Institute at Bethesda, Maryland, Barnard Free Skin and Cancer Hospital and Washington University at St. Louis, Lankenau Hospital at Philadelphia, Memorial Hospital in New York City, University of Minnesota, Yale University, University of Texas, University of Wisconsin, Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine, Rockefeller Institutes for Medical Research in New York and Princeton, Chicago Tumor Institute, Michael Reese Hospital in Chicago, and in other universities and hospitals and in the research laboratories of several industrial organizations.

Certain wartime researches, notably those associated with atomic fission and chemical warfare, have yielded information and materials which can be applied directly to the cancer problem. The production of radioactive tracer substances (isotopes), such as radioactive carbon, phosphorus, iodine, sulfur, sodium, chlorine and iron, some of which were available before the war, has provided new tools for studying the metabolism of cancer cells, as well as new therapeutic weapons for the control of the disease. The advent of uranium-graphite atomic piles has greatly enhanced the manufacture of these and other tracer substances, formerly made with the cyclotron. Similarly, nitrogen mustard compounds, developed during the search for war gases by the Army's Chemical Warfare Service, have been found to exert a profound effect upon the blood cell forming tissues of the body; intensive investigation is now underway to determine the effects of these war-inspired compounds upon leukemia, lymphosarcoma and Hodgkin's disease. These constitute examples of the ways in which the fruits of war research may lead to the preservation rather than to the annihilation of man. A large proportion of the time spent in making this survey has been devoted to visiting various cancer research laboratories for the purpose of obtaining first-hand information from the persons directly engaged in such work.

* Received for publication, April 15, 1947.

Charles F. Kettering and I have had a particular interest in the work accomplished since 1939 at the Barnard Free Skin and Cancer Hospital at St. Louis, under the direction of E. V. Cowdry. Three recent visits have been made to that institution. Scientists engaged in cancer research are enthusiastic in their praise of Cowdry's "straight-down-the-road" program of determining quantitatively the changes which occur in the skin cells as cancer develops following the application of a chemically pure carcinogen, methylcholanthrene. In the *Annual Review of Biochemistry for 1945*, in the chapter on "The Biochemistry of Malignant Tissues", Jesse P. Greenstein, of the National Cancer Institute, made the following statement: "It is apparent that at the present time each approach has illuminated the problem of malignancy without as yet penetrating to its core. We know that a wide variety of agents will produce a tumor and that the one formed is metabolically very much like all other tumors. The core of the problem lies in the nature of the malignant transformation. So far, we know reasonably well only states A and Z, *i.e.*, the fully normal and the frankly malignant. Recognition of such states as H, M, and P, as exemplified among others by the valuable series of studies by Cowdry's group on epidermal carcinogenesis, should be one of the future aims of investigation."

Cowdry's approach to the problem of what happens within a cell when it is transformed from a normal to a cancerous state is unique in many respects. His results have demonstrated that a single institution can, to advantage, concentrate all its efforts over a period of years on one sound and carefully chosen problem, and that the program and the goal can remain unaltered despite changes, from time to time, in the membership of the team. One important advance has been the determination by fluorescence microscopy and spectrography of the parts of the skin which take up the carcinogenic substance, methylcholanthrene. The latter phase of the program was accomplished at Antioch College by collaboration of Drs. Knorr, Albers and Rothmund of the Kettering Foundation for the Study of Chlorophyll and Photosynthesis. This work, interrupted by the war, has now been resumed. One step in this collaboration has been the utilization by Rothmund of the radioactive isotope of carbon, C14, in the synthesis of methylcholanthrene. A natural outcome of the work done thus far in determining the fate of the carcinogen in the living body cells has been the recognition of the necessity for identifying the physicochemical changes produced within living cells which cause them to become cancerous. Successful attempts are now being made to separate the formed elements of the epidermal cells by differential ultra-centrifugation. It has been possible to isolate the nuclei and several formed elements of the cytoplasm of both normal and cancer cells by this means. The Barnard workers have also made the important observation that a new chemical equilibrium occurs within ten days following the application of the carcinogen to the skin cells; the content of calcium, copper, iron, cholesterol and biotin is reduced by approximately fifty per cent within ten days of the initial application of the carcinogen. The observation that anhydrous lanolin inhibits the production of cancer when methylcholanthrene is applied in lanolin rather than in benzene has led to a further search for the actual in-

hibiting substance contained in lanolin. The knowledge that malignant tumors have a high nucleic acid content and that the cellular nucleoproteins are concerned with the rate of growth of cells points the way to the next step in the program of investigation at the Barnard Hospital. In furthering these observations, direct collaboration has been established with Caspersson, of Sweden, in utilizing ultraviolet spectrophotometric and cytochemical methods for obtaining quantitative data on the part played by nucleic acids in tumor production. Caspersson pursued these fundamental studies with great vigor during the long six years of war. Having determined certain chemical, physical and morphologic changes which occur in the whole epidermis at the site of cancer production with the carcinogen, it now becomes of urgent importance to determine the biochemical and biophysical changes which take place *inside* individual living body cells.

The National Cancer Institute at Bethesda, Maryland, was the first institution visited. This institute was created by an act of Congress, in 1937, and represents an important federal plan of direct and supportive cancer research. The grant-in-aid program has made funds available to numerous institutions and workers in cancer and has assured them of *long-term support*. The staff of the laboratories at Bethesda is composed of leading groups of scientists who are widely representative of many basic disciplines and who are engaged full-time in cancer research. During the past five years, despite interruptions occasioned by war, several noteworthy advances have been recorded. The work of Heston on the definite rôle of specific genes in susceptibility to lung tumors has thrown new light on the factor of heredity in cancer. Earle has observed normal connective tissue cells, grown in test tubes and exposed to a carcinogenic compound for many generations, undergo transformation into malignant cells. The contributions of Andervont continue to be of fundamental importance, particularly as they augment and confirm much of Bittner's work at Minnesota. Greenstein and his co-workers have systematically studied a whole range of enzymes in normal tissues and their neoplastic counterparts. The program of the National Cancer Institute has been expanded since the termination of the war. A comprehensive search for chemicals showing destructive effect on tumors is being vigorously pursued by its chemotherapy group, led by Shear. An epidemiologic unit is being established at Harvard, and a clinical research unit is being developed at the University of California Medical School in San Francisco, directed by Michael B. Shimkin, Surgeon, U. S. Public Health Service.

Reuben L. Kahn, of the University of Michigan, the originator of the test for syphilis which bears his name, is now devoting his full energies to the immunologic aspects of cancer in laboratory animals, in collaboration with Cowdry in St. Louis, Earle in Bethesda and Little in Bar Harbor. His lifetime work in tissue immunity fits him for exploration of this phase of the cancer problem.

The other cancer research laboratories visited thus far include: the Institute for Cancer Research at the Lankenau Hospital, Philadelphia; the Memorial Hospital, New York City; the Atypical Growth Research Unit at Yale University

School of Medicine; the Department of Cancer Biology at the University of Minnesota; Northwestern University; the University of Wisconsin; the University of Michigan; the Chicago Tumor Institute; the University of California Medical Schools at San Francisco and Los Angeles; the Los Angeles Tumor Institute; and the newly created Research Institute of the Cedars of Lebanon Hospital at Los Angeles, directed by Dr. Harry Goldblatt. Important work is being done at several other universities and hospitals and visits to these research centers will be made in the near future. In addition, an attempt is being made to gather and correlate the immense accumulation of world literature on the cancer problem published during the war years in a great variety of medical and nonmedical, but related, technical journals.

For brevity's sake no attempt will be made at this time to present in detail any recitation of the greatly varied attacks being made on the cancer problem in the different institutions visited. It suffices to say that they involve many disciplines, such as biology, physics, chemistry, botany, genetics, mutations, bacteriology, protozoology, pathology, tissue culture, virology, radiology, enzymology, cytology, cytochemistry, and the study of endocrinology, physiology and pharmacology, nutrition, vitamins, proteins and steroids. The unity of all science is revealed by the unmistakable manner in which these artificially separated fields of endeavor overlap one another. The recently constituted Committee on Growth of the National Research Council and the American Cancer Society have set up twenty separate research panels, each concerned with some discipline related to the broad attack on cancer. In a problem so complex and so diffuse, the great necessity is to provide the means whereby the hosts of workers in the various fields related to cancer research may work in close harmony with each other.

The attack on cancer through research is, of course, not limited to the United States, and was continued under adverse conditions during the war in England, France, Germany and the Soviet Union. The great team of chemists in England who discovered the first of the pure carcinogenic chemicals is now engaged primarily in synthesizing and testing chemicals having possible therapeutic effects on tumors. The efforts of these men already have been partly rewarded by the work on stilbene compounds by Dodds, and on the effects of urethane in leukemia by Haddow and his group. A grant for a study of the rôle of viruses in cancer has been extended to the Imperial Cancer Research Fund. Lacasagne, one of the pioneers in the study of the rôle of hormones in cancer, has continued his work in Paris. Many reports, particularly on biochemical aspects of the problem, are becoming available from the German literature of the war years. In the Soviet Union, the Oncology Institute in Leningrad certainly deserves recognition as one of the outstanding institutions devoted to the study of cancer. In Moscow, the newly created Academy of Medical Sciences has made cancer one of its most important problems. Shabad's discovery of carcinogenic substances in the liver and other tissues of man is of great interest. Roskin and Klueva have reported that lysates of *Trypanosoma cruzi*, the causative organism of the dreaded Chagas' disease, may have a selective action on

malignant tumors. Medical science knows no international boundaries and cancer has no respect for differing political ideologies or for arbitrary geographic boundaries. The work of our Russian colleagues is and will continue to be important, and information regarding it is considered essential.

WHAT CAN BE DONE TO CONTROL CANCER NOW

According to the information issued by the National Office of Vital Statistics of the U. S. Public Health Service, 177,464 persons died of cancer in the United States during 1945. Taking into account the inevitable errors which prevail in collecting vital statistics, particularly when it is known that diagnoses are confirmed by postmortem examination in hardly ten per cent of cases, it is probably justifiable to state that more than 185,000 persons now die of cancer in this country every year. Since it is believed that for every death from cancer there are five active cases of cancer, it is estimated that more than 800,000 persons are now suffering from this disease in the United States. It is further estimated that, at the present rate of mortality, roughly 15,000,000 persons now living in the United States, or one out of every nine, are doomed to die of cancer unless means of control is discovered. Nor is the gravity of the situation to be measured by the sickness and death rates alone. Cancer differs from most other diseases in that death from it is preceded by a long-lasting debilitating illness. Economically, it affects not only its victims but the entire family for many months. It may remove the breadwinner from gainful employment, or disrupt the family by afflicting the wife and mother. To the loss of income must be added the immense cost of medical care. Even more important than this economic loss is the immeasurable human suffering and anguish caused by cancer.

The weapons now at hand are: (1) surgery, (2) radiation in the form of x-ray and radium, and (3) education of lay people and their doctors in the early detection of the accessible forms of cancer, so that the patient may be given the full benefit of surgery or radiation in the early stages of the disease when these measures are effective. Dr. Ludvig Hektoen has made the challenging statement that if all accessible cancer were treated early by surgery or radiation, or both, 50,000 lives could be saved yearly. As an example of life-saving now possible by the use of new radiation weapons, Dr. Max Cutler of the Chicago Tumor Institute has reported three-year and five-year "cures" by high voltage irradiation treatment of cancer of the larynx, in all stages of the disease except where there was total fixation of the laryngeal structures. The five-year "cure" rate stands at 48 per cent, with recovery of speech. Prior to 1922, the chief hope of cure was in surgical removal of the larynx, with a resultant loss of speech in most cases.

Nationwide success in recognizing cancer in its early phase depends on the establishment of well staffed cancer detection clinics in every first-class hospital in the country. The gains to be made in such a program are already becoming manifest by the results obtained in the detection clinics at Philadelphia, Detroit, Chicago, Los Angeles and New York.

Equally important is the training of a corps of surgeons in the peculiar, often heroic, requirements of cancer surgery, and of a corps of precision radiologists, together with the development of supervoltage x-ray equipment such as the new 2,000,000 volt apparatus and the betatron (10,000,000 to 100,000,000 volts).

In the past few years, surgeons have dared to attempt operations formerly considered impossible and are saving lives from cancer hitherto considered inoperable, such as cancer of the lung, brain and esophagus. This has been made possible by the development of new methods to combat shock, safer anesthesia, and the adoption of new methods of intravenous nutrition. As with radiation therapy, this advanced type of cancer surgery should be practiced in every first-class hospital in conjunction with cancer detection centers.

In summary, the saving of 50,000 lives annually which is now possible, urgently demands the training of a new corps of diagnosticians, surgeons and radiologists schooled in the problems peculiar to cancer.

PRESENT RESEARCH ON THE NATURE OF CANCER AS BEARING ON ITS CAUSE, PREVENTION AND CURE

Although the problem of cancer is as old as recorded history, modern experimental cancer research is new. More progress has been made during the past fifty years than during the previous 5000 years. Real progress began only about fifty years ago, when Hanau and Jensen first demonstrated the successful transplantation of tumors from one animal to another. Japanese workers, some thirty years ago, first produced cancer experimentally in animals by repeatedly rubbing tar on the ears of rabbits.

Thirteen years ago, English workers succeeded in isolating from tar a definite organic chemical (3,4-benzpyrene) which produced cancer in mice. This led to the artificial synthesis of a wide range of organic cancer-producing compounds, the fundamental component of many of which is anthracene. Some 1028 compounds have now been tested for cancer-producing activity, of which 284 have been found more or less active in the production of cancer in animals.

One of the most powerful of these carcinogenic compounds, and the most useful to date, is methylcholanthrene. It may be quite significant that this compound is closely related to, and can be produced from, cholic acid which is a normal constituent of human bile. It is also closely related chemically to the sterols, of which both the male and female sex hormones are examples. Similarly, the chemical structure of steroids and certain vitamins closely resemble certain known chemical carcinogens. The discovery of chemical carcinogens has not thus far thrown direct light on the causation of human cancer. They have served, up until now, as tools for the production of experimental cancers in animals. Since methylcholanthrene is a close chemical relative of cholic acid, the question naturally arises whether or not a slight shift in the chemical structure of naturally occurring cholic acid might give rise to a carcinogen similar to methylcholanthrene being responsible for the causation of human cancer. Similarly, many chemical carcinogens produced in the test tube have a strikingly intimate relationship to other substances which occur naturally in the

human economy. The shifting of the position of a single double bond will convert some highly carcinogenic hydrocarbon compounds into wholly innocuous ones, and *vice versa*.

While the carcinogens have as yet thrown little light on the cause of human cancer, their close relatives, the sterols, in the form of male and female sex hormones, are beginning to give a hint of the possible future control of certain types of human cancer. The outstanding example of this is derived from the discovery of Huggins that castration in the male definitely checks the progress of cancer of the prostate gland in a high proportion of cases. This effect is apparently due to the removal of the chief source of male hormone from the body. This hormone mediates the growth of the human prostate and also apparently encourages the growth of prostatic cancer cells which are derived from normal prostate tissue. It has now been demonstrated that it is not necessary to castrate in order to produce this effect. Female sex hormones, such as ethinyl estradiol or diethylstilbestrol, have just as great, if not a greater retarding action on prostatic cancer. The work of Huggins contradicts and casts doubt on the old teaching that cancer cells are unrestrained anarchists in the body economy, and that cancer is an irreversible, steady progressive growth.

Other recent work raises the question of whether hormone administration may not be used effectively in an effort to prevent cancer of certain types. Heiman, of Columbia University, has notably reduced the occurrence of spontaneous breast cancer in inbred mice highly susceptible to cancer of the breast by injecting them with hormones antagonistic to the female hormone. He is now beginning an actual attempt to prevent human breast cancer by the systematic injection of these substances in women who have a family history of cancer of the breast. In certain strains of mice, the long continued administration of massive doses of female hormones has given rise to cancer in the breast, uterus, testicles, bones or lymphoid tissue. Cancer of the breast in males often yields to orchidectomy or to silbesterol therapy. Similarly, Adair of the Memorial Hospital, New York, and others, have found that large doses of the male hormone, testosterone, often produce a beneficial effect on bone metastases of breast carcinoma. These observations are overshadowed by another which may have far greater significance, namely, that the age of greatest susceptibility to cancer in men and women strikingly coincides with the period of progressive, physiologic (and sometimes pathologic) deficiency of hormones. Here, certainly, is a fruitful field for intensive investigation, in the clinic as well as in the laboratory.

In addition to the carcinogenic hydrocarbons and the sterols, a large variety of chemical compounds of entirely different composition, all the way from aniline derivatives to sugars and even simple acids, have produced cancer in experimental animals. The very fact that such an enormous number of entirely unrelated chemical compounds has been incriminated as inciters of cancer leads to the suspicion that none of them is to be considered as the over-all primary cause of the cancer process. One or several of these compounds may play a chemical rôle in the causation of cancer, but some other factor or factors

must also operate. A lead as to what these other factors may be is perhaps supplied by the observations on breast tumors in mice.

Of all the work done in experimental cancer research, the greatest amount has involved cancer of the breast in mice. Three special characteristics of this type of cancer may be mentioned:

1. Breast cancer of mice arises most frequently in strains inbred to bring out the factor of inherited susceptibility. Thus, by inbreeding and selection, one can produce at will strains of mice which are highly susceptible, or strains which are highly resistant to the apparently spontaneous occurrence of breast cancer.

2. There must also be present the growth-stimulating effect on the breast tissue of ovarian and pituitary hormones; otherwise cancer of the breast will not occur, even in highly susceptible inbred strains.

3. The third requirement is the presence of an agent, known to be contained in the milk of the mother mouse. The classic work of Bittner has shown that a large percentage of normally low-cancer strain mice will develop cancer of the breast when they are foster-fed by a high-cancer strain mother. Conversely, when the offspring of a high-cancer strain mother are removed by cesarean section to prevent them from suckling from her, and are foster-fed by a mother from a strain in which cancer is practically nonexistent, the expected occurrence of cancer in these offspring does not take place.

The discovery that some cancer inciter is transmitted in the milk of the mother mouse marks a major advance in modern cancer research. What is the nature of this cancer-producing agent? For some ten years this inciter was known as the Bittner milk influence, the Bittner milk factor, or the transmissible agent. In March, 1946, Dr. Robert G. Green of the University of Minnesota, reported his observation that the Bittner transmissible agent is probably a virus.

The evidence that the Bittner agent contained in the mother's milk of mice with breast cancer is a virus, as recently advanced by Green, Bittner, Mosesey and Barnum, may be summarized as follows: (1) by homogenization and ultra-high speed centrifugation, cancer cells in the breast of the mouse can be completely disrupted, with a resultant yield of sub-microscopic particles of virus size; (2) these cancer tissue centrifugates from the cancer of the breast of the mouse, injected into rabbits and rats, produce an antiserum which is not specific against normal mouse breast tissue, but is specific against mouse cancer cells and their products; (3) when the mouse cancer centrifugate (virus) is mixed with the anti-mouse cancer serum, the virus is inactivated so that it no longer produces breast cancer in mice; and (4) when the rabbit anti-mouse cancer serum is mixed with mouse breast cancer cells, it kills those cells so that they are no longer capable of producing transplantable breast cancers when injected into mice.

It is Green's belief that the cancer virus, penetrating into the normal mouse breast tissue cells, so modifies them chemically that they lose their species identity, are freed from normal growth controls, and then are able to multiply without limit, leading to the ultimate destruction of the animal. This work of Green's puts a new interpretation upon the earlier observations regarding the

virus causation of leucosis in chickens (Ellerman and Bang), the sarcoma of chickens (Rous), the virus papilloma of Western cottontail rabbits (Shope), and the adenocarcinoma of the kidneys of leopard frogs (Lucké). It has been known for many years that these tumors of chickens, rabbits and frogs are caused by viruses.

If Green's results with cancer of the breast of the mouse can be confirmed for human cancer, the hope for the control of human cancer becomes far greater than heretofore. If human cancer, in general, is caused wholly or in part by a virus, this would remove the baffling and frustrating conception that cancers arise by some mysterious chemical spontaneity within the human body. It would mean, on the contrary, that the cause of cancer is a living entity, which enters the body from without. Should this prove to be so, we are now, in relation to cancer, in a position identical with that of Pasteur when he dispelled the fallacy of spontaneous generation and proved that microbes, entering the human body from without, caused infectious diseases. Furthermore, the hope for the control of cancer would become much greater, as there is the possibility of keeping the infective agent from entering the body, of vaccinating against it, or of attacking the disease with a specific antiserum, once it has started. Finally, there is the hope that some new chemotherapeutic agent, or some antibiotic on the order of penicillin or streptomycin, may be found which will destroy the cancer virus.

Roger J. Williams and his associates at the Biochemical Institute of the University of Texas have made the significant observation that there is a specifically different chemical pattern in cancer cells as compared to normal cells in respect to their content of certain B vitamins (thiamin, riboflavin, niacin, pantothenic acid and pyridoxine). The quantitative demonstration of differences between the chemical constituents of normal versus cancer cells emphasizes anew the urgent necessity for intensified study of the physicochemical differences between cancer cells and normal cells. Far from discouraging investigations characterizing the physicochemical changes *within the living cell*, the accumulating evidence in favor of a virus origin of at least some forms of cancer makes more urgent than ever the expansion of such physicochemical studies of the cancer cell, and of cell growth in general. The fruits of such researches would apply not only to cancer but to the whole gamut of degenerative diseases, which are now responsible for most deaths in man.

While diligent search is being made for specific causes of cancer and for a clearer understanding of the physicochemical changes which characterize the cancer cell, every effort must also be made to discover substances which will inhibit or destroy cancer cells without harming the normal body cells. The important observations of Shear, of the National Cancer Institute, and Reimann, of the Lankenau Hospital Research Institute, concerning the destructive effect of a bacterial polysaccharide (derived from filtrates of cultures of *Bacillus prodigiosus*, on the order of "Coley's toxins") on tumors of mice have led to cautious trial of this substance in human subjects with advanced cancer. Four patients with fibrosarcoma appeared to have been distinctly benefited: the

course of the disease was not appreciably altered in 21 additional patients suffering from other types of malignant disease.

COMMENT

Never has the need been greater for integrated teamwork among workers in all the disciplines which have so far contributed so much in cancer research. In this connection, it is appropriate to quote from a published statement made by Roger J. Williams: "I should like to express high optimism with respect to cancer research, for the simple reason that investigators are ceasing to follow the 'hunch' method, which causes each investigator to play a lone hand, and are adopting more scientific methods, which not only get down to fundamentals, but also allow for cooperation and collaboration in the larger sense. No single investigator can do much alone, but with a small army of investigators who understand one another and can use one another's results, the future looks bright."

The likelihood that the whole solution of the riddle of cancer will be found ultimately by one individual, by one group in a single institution, or by one organization, is remote. As in most fields of intellectual endeavor, the day of lone-wolf researchers is largely ended, except as members of a team. Idea men are, of course, the key men in any group research. Thus far, no Pasteur or Edison has appeared to dominate cancer research. The challenge is great enough to demand expansion and integration on nothing less than a world-wide scale.

We are undoubtedly on the verge of great developments in cancer research. The interrelationships of viruses, intracellular enzyme systems and mitochondria; the study of cancerolytic substances produced by living microorganisms and by bacterial products; endocrine physiology; steroid metabolism; the identification of transmissible parental factors other than genetic; and the mechanism of gene mutations are now being vigorously attacked. The belief is gaining strength that enzymes are "organic catalysts" which determine intracellular chemical behavior and growth, normal and abnormal. Isotopic tracer substances are now being widely employed as tools in biochemical and biophysical researches in cancerous and noncancerous animals and in man.

Thus far, most American, British and French cancer research has been conducted on a "cup-in-hand", "hit-or-miss" basis. The salaries paid to cancer workers have been unattractively low and there has been little assurance of the long tenure which the job demands. The reorganized American Cancer Society's laudable enterprise in conducting annual nationwide fund-raising campaigns is an important first step in the right direction and so is the development of large cancer research centers.

The problem is so vast and so challenging, however, that substantial additional and continuous federal support is also urgently required. Endowments, private gifts and bequests are to be hoped for, but such sources are drying up because of the current income and inheritance taxation practices and falling interest rates on investments. To quote Dean Stafford Warren, of the University of Cali-

ifornia Medical School at Los Angeles: "The essential requirements for a successful cancer research program are fully adequate resources, both of men and money, plus continuity of full-time effort." The panel on cytochemistry of the National Research Council alone has requested an annual allotment of \$500,000 to support and to expand promising researches in this vital phase of cancer research in already existing laboratories.

The pending Taft-Smith-Ball-Donnell Health Bill now contains a provision authorizing a separate indefinitely continued annual appropriation of \$10,000,000 to enable the Surgeon General of the U. S. Public Health Service to develop more effective measures for the prevention and control of cancer, through grants-in-aid to states, counties, health districts and other political subdivisions of the states. This feature of the bill will undoubtedly receive hearty support from most of those engaged in cancer research.

Enough enthusiastic young scientists, in enough places, supplied with enough money, fired with common zeal to do the job on a world-wide scale is the goal. Much of the cancer research of the past has been done on a part-time avocation basis by harried professors with heavy administrative and teaching duties and by their short-term graduate students striving to complete a thesis for a doctorate. The newly constituted World Health Organization of the United Nations of which Surgeon General Thomas Parran, of the U. S. Public Health Service, is the president, will be the logical clearing house for the interchange of cancer research information. The Fourth International Cancer Congress, to be held in St. Louis, September 2 to 7, 1947, will provide a new and wonderful opportunity for the correlation and interchange of information by cancer researchers from all parts of the world.

America must lead the way in cancer research, as in other post-war endeavors. The public conscience is finally sufficiently aroused to demand a solution to the problem. Certainly the discovery of the causes and the cure of cancer is equal in importance to the production of the atomic bomb which cost two billion American dollars, and two and one-half years of intensive work by 100,000 American workers. By such discovery, we may counter "man's *destruction* of man" with "man's *salvation* of man".

THE PATHOLOGIST'S CONTRIBUTION TO THE CLINICAL INTERPRETATION OF DISORDERS OF THE NEWBORN*

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For the anatomic pathologist who is accustomed to the multiplicity of major pathologic changes commonly found on postmortem examination of the adult, the meager evidence left by disturbances responsible for death in the newborn often seem of little interest.

The hold on life is very tenuous during the hours of birth and the time immediately thereafter, and even a slight shift may swing the balance in either direction, to life or to death. In the older child and the adult, the hold on life is strong and, except for those who die of sudden fulminating infections or of trauma, major alterations in function have usually existed for a sufficient length of time to produce extensive lesions in one or more vital organs before death actually occurs.

As life expectancy increases and more people live to an older age, a greater number of deaths can be expected to occur in association with degenerative changes, and the pathologic lesions associated with them may become more and more striking. Before the time when the degenerative diseases begin to make themselves manifest, the death rate is low and the majority of the deaths that do occur are a result of accident or infection.

During intra-uterine life and in the period of adjustment to an independent existence, a group of disturbances not seen in later life is responsible for failure to survive. Frequently these disturbances are of such brief duration that they leave little or no demonstrable alteration in tissue structure and, from post-mortem examination alone, it is often difficult to reconstruct the events which transpired to cause death.

The study of diseases of the newborn is not of recent origin, but it has met with many vicissitudes and has advanced much more slowly than would be expected from the proportionately high mortality that occurs at this age period. Perhaps this has been partly due to the greater economic value of the mother, father and older children and to the seeming cheapness of infant life. Through the ages, in crowded areas or in other locations where food was scarce and life precarious, the death of an infant from neglect or by direct intent meant one less child to feed and one less person to become a competitor in the struggle for existence. Even in civilized countries in relatively modern times, the death of a newborn infant has not been considered too important and has been dismissed largely as an "act of God" about which little could be done.

It has been intimated, even in relatively recent times, that it is useless to

* Presidential address read at the Chicago Pathological Society, October 14, 1946.
Received for publication, April 16, 1947.

attempt to decrease the infant death rate because by so doing an immediate burden, which can bring no ultimate return, is placed on the community. It has been thought that the death rate under two years is established at a fairly constant level and that, by decreasing mortality during the fetal and neonatal periods, a compensatory increase would occur later. A certain percentage of infants was believed to be unfit and destined to die, and it was considered better to permit them to succumb early, than to live a few months at the expense of those who were caring for them.

In spite of a somewhat general disinterest in the fate of the newborn infant, one particular phase of newborn pathology has been of widespread interest for many centuries, and probably for as long a time as man has been in existence. The monstrosities that occur as a result of abnormal intra-uterine development, or inhibition or alteration of the usual embryologic processes, have been important to the parents and often to the localities in which they were born. The mother who gave birth to a monstrous infant was frequently ostracized from the community or even destroyed along with her child, for it was often believed that only by being bewitched or having cohabited with a beast or the devil could such a creature have been conceived.

In the middle of the last century, there were several French, German and Italian anatomists who described and catalogued innumerable varieties of malformations and in the more learned works of today many of their ponderous terms are still used. Taruffi, with 4000 pages published in eight volumes over a period of fourteen years, St. Hillaire and Dareste, are only a few.

The pathology of the fetus and newborn, however, in the minds of many people consists too largely of teratology, and because of the seeming uselessness of this field the subject has not aroused much interest, nor has it developed to the extent that might otherwise have been possible.

The first work on fetal disease, as distinguished from monstrosities, was *De Morbis Foetuum in Utero Materno*, which was presented as a monograph and publicly supported by Phillipus Jacobus Düttel in 1702. In his preface he began, "So wretched and miserable is the condition of mankind that not only are men tormented by innumerable ills throughout their lives, but foetuses also are not free from evils and sicknesses whilst they are still shut up within the prison of the womb, and before they breathe with joy the vital air and look upon the light." He described fetal variola, morbilli and syphilis. He discussed jaundice in the mother and fetus, mentioned a case of generalized dropsy, and appeared to have believed that exomphalos was due to disease. He commented that no fetal disease was more common than epilepsy and described an illness known as intermittent fetal fever. These conditions seem to have been all that were known at that time, and the latter part of his work is devoted to a consideration of the cause of fetal disease.

In the intervening 240 years occasional publications dealing with this subject have appeared. During the latter half of the last century, several books were published which gave accounts of the anatomic and physiologic development of the embryo and fetus and of some of the pathologic disturbances to which it

was subject. Ballantyne felt very keenly the need for some organization in the field of pathology of the newborn and published several volumes during the late 1890's and early 1900's. The first was entitled, *The Diseases and Deformities of the Foetus: An Attempt Towards a System of Ante-Natal Pathology*. It included an excellent summary of the rather extensive literature that had accumulated in the field of fetal and neonatal disease up to that time and presented various systems of classification that had been used by different investigators. The remaining two-thirds of the book was taken up by a discussion of "general dropsy of the fetus". In 1895, the second volume appeared, and this was devoted to a discussion of the diseases of the skin and subcutaneous tissues. A somewhat more comprehensive *Manual of Ante-Natal Pathology and Hygiene* dealing with diseases of the fetus was published in 1902 and the better known second volume by the same title, and dealing with the embryo, appeared in 1904. Simultaneously with the production of these works Ballantyne edited "*Teratologia: A Quarterly Journal of Antenatal Pathology with Reviews of the Current Literature on the Subject*". This seems to have been relatively short-lived and appeared for only a few years.

Birnbaum, in 1909, published a book entitled, *Malformations and Congenital Diseases*, which was translated from the original German into English. It is little known and few copies are available. Since then no major work has appeared, although various phases have been discussed in several books and in many brief papers.

The Medical Research Council in Great Britain financed a comprehensive study of the causes of stillbirth and early infant death in the early 1920's. This included the postmortem examination of more than 1000 fetuses and infants in centers in different parts of England. The results of these autopsies together with the histories of the mothers and of the infants up to the time of death were reviewed and the material published in 1922 by Eardley Holland and Janet Lane-Capon.

To the average general practitioner today, and to the majority of the medical men delivering babies and caring for them in the early days of life, the reasons why infants die, either before or after birth, are a matter of almost complete mystery. In the absence of an autopsy, and even when one is performed, the term "intra-uterine asphyxia" is given as the cause of death of most stillbirths and "prematurity" or atelectasis" is used to explain the death of most infants who were born alive.

As was mentioned previously, the average pathologist is interested in spectacular or unusual changes and when, on examining the bodies of several infants, he finds a few petechial hemorrhages beneath the parietal and visceral pleura, a mild increase in fluid beneath the meninges, an increase in the size of the spleen, or perhaps nothing at all, he finds it convenient to turn the postmortem examination of these infants over to the youngest member of his group, often one who is considered not yet adequate to investigate adult material.

The postmortem examination of the newborn infant is a much easier physical task than examination of the adult, but at that point the element of greater ease ends. To observe, to interpret, and to make the interpretation of value to the clinician, requires skill equal to that demanded by any field of pathology.

It is not enough to list the postmortem findings and to have them typed and filed. The obstetrician and the pediatrician want to know how these findings are to be interpreted in relation to the death of the particular infant in question and how to prevent them from occurring in other children.

The fetus that dies *in utero* has never been observed as an individual, and in most of the infants who die in the early days of life, the period of clinical observation is brief, and the variations in their symptoms are very slight. It is the pathologist who must review the history, who must observe the residual changes that still exist in the body of the infant and who must reconstruct the course of events. To make any autopsy worthwhile, a history should be available, and this is especially important when dealing with subjects in this age group. At no other time is close cooperation between the clinician and the pathologist more essential.

A well trained general pathologist should have a good medical background and, ideally, an extended practical experience in the diagnosis and treatment of living patients. So, also, the pathologist examining the body of a dead infant should have had experience in the actual clinical problems of obstetrics and pediatrics and should have a continuing interest in and contact with clinical problems. Our modern trend to early specialization often makes such overlapping of clinical and laboratory fields seem difficult or impossible, and both specialties may suffer in consequence.

The pathologist has an ideal opportunity to study both the origin and the end of an abnormal process and to be the principal stimulus for the furtherance of the understanding of the problems of the newborn. There is still much that is not understood, but the fields in which causative agents are recognized are constantly broadening. This accumulating knowledge constitutes a definite factor in bringing about the reduction in infant mortality that is becoming generally apparent. One of the chief functions of the pathologist is to make the pediatrician and obstetrician aware of the variety of pathologic states which may exist in these early days of life.

In Chicago, in 1915, the death rate under one year of age was 125 per 1000 births. Thirty years later, in 1945, this had been reduced to less than 30 per 1000; a drop to a figure less than one-fourth of its original level. The total mortality at the Chicago Lying-In Hospital for all infants and fetuses weighing in excess of 400 gm. at birth has decreased from 48 in 1936 to 28 in 1946.

What kills infants in this age period and what is responsible for this decrease? The four leading causes of death in the combined groups of stillbirths and neonatal deaths are anoxia, malformations, birth trauma and erythroblastosis, while in the liveborn group prematurity plays an extremely important rôle. Syphilis and acute infections have been largely eradicated in some localities, but in others, they still make an important contribution to the total mortality.

Observation of infants in the nursery by the pathologist makes it possible after a while for him to predict with considerable accuracy what the pathologic lesions will be in an infant that succumbs. This is of no help to that specific infant and, in some instances, it does no good for succeeding infants who show the same symptoms. If, however, one is aware of the pathologic changes that are

present in relation to certain symptoms, there is a good chance that a cure, or a means of prevention, eventually can be found.

Infants delivered by cesarean section, who seem normal at birth, but who subsequently develop progressive respiratory distress and die at from ten to fourteen hours of age, frequently have an increase in fluid in the meningeal spaces and a progressive resorption atelectasis.

Infants, moderately premature, who are a little slow to breathe at birth and who became irregularly dyspneic and cyanotic, frequently develop costal retraction, show an increasing struggle for breath and who die from twenty-four to thirty-six hours of age often have abnormal findings limited to the lungs. The lungs show a liver-like consistency due to capillary congestion, resorption of air and the presence of a hyaline membrane surrounding the few remaining air spaces.

Infants born with subcutaneous ecchymoses, mild edema, enlargement of spleen and liver, often die in a few hours and exhibit the extramedullary erythropoiesis characteristic of erythroblastosis.

Infants who look peculiar at birth and who have an increase in inter-pupillary measurement, a prominent epicanthic fold, a prominent cleft between the lower lip and the chin, large low-set ears, often die from five to ten hours after birth with complete renal agenesis.

Infants who appear normal at birth and who, on the second or third day during bathing or feeding, suddenly go into collapse and die within a few minutes, often are found to have ruptured subcapsular hematomas of the liver.

Infants who appear normal at birth, with heart beating vigorously, but who make a few unsuccessful attempts to breathe and never breathe again despite repeated attempts at resuscitation, may fail to breathe because the chest is occupied by a large heart, by the viscera from the abdominal cavity, or is compressed by upward pressure from polycystic kidneys. The trachea may be closed by a cartilaginous malformation.

SUMMARY

There is a remarkably close correlation between the clinical course of infants during the newborn period and the findings present on postmortem examination. The pathologist can be of immeasurable help to the clinician in reconstructing the course of events leading to death in the newborn period or during the course of labor.

To be able to combat a disease, the disease must be known. The frequently repeated statement that the majority of infants die for unknown reasons is untrue. Rarely is there an infant for whom a cause of death cannot be found by combined study of clinical and pathologic data.

The particular fields yet to be conquered in the prevention of death in this age period center about premature delivery, ante-partum intra-uterine death and malformations. The study of the diseases of the fetus and newborn has shown great progress in the last 250 years, but there is still much to be learned.

SERUM PHOSPHATASE AND OTHER TESTS OF LIVER FUNCTION IN INFECTIOUS MONONUCLEOSIS*

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Infectious mononucleosis is not infrequently accompanied by jaundice.^{3, 5, 7, 25} Some have attributed the jaundice to compression of major bile ducts by enlarged lymph nodes, others to the coincidental occurrence of epidemic hepatitis and mononucleosis and still others have suggested an identity of the two diseases. The nature of the lymph node lesion⁹ is such that the likelihood of compression of bile ducts would appear remote. The observation of abnormal lymphocytes in the blood smears of patients with epidemic hepatitis constitutes neither a constant nor a characteristic feature of this disease and the course and findings of the condition have little similarity to those of infectious mononucleosis. Recent studies^{2, 12, 16, 24, 27} indicate, moreover, that in mononucleosis the liver is the seat of a lesion which differs from that of epidemic hepatitis and is probably related to the cellular reaction occurring elsewhere in this disease.

Liver enlargement, both with and without jaundice, is often observed in infectious mononucleosis and it is probable that the liver is affected far more frequently than has been appreciated. This is borne out in the studies of 15 cases of mononucleosis without jaundice reported by Cohn and Lidman.⁶ In this group, all cases manifested abnormalities in one or more tests of liver function.

The present study was carried out as the result of the accidental observation of an elevated serum phosphatase in a nonjaundiced patient with infectious mononucleosis (Case 1). Subsequently, 33 additional patients with this disease were encountered serially and were investigated with a view to determining the frequency with which phosphatase alteration occurred in infectious mononucleosis. In the course of the study, other tests related to liver function, such as icterus index, cephalin flocculation and thymol turbidity, were also carried out. All 34 patients showed the blood pictures and other clinical manifestations considered representative of infectious mononucleosis and all but one had heterophil antibody titers of at least 1:112, a diagnostic level.²⁵ Only one patient was frankly jaundiced and two others exhibited a "subicteric" hue.

Since the patients were studied in several different hospitals, the methods for estimating serum alkaline phosphatase varied with the laboratory in which the procedure was carried out. In 28 instances, the King-Armstrong method¹³ was used and, in the remainder, the Bodansky procedure was followed.⁴ The maximum adult normal level for the former method was given as 13 units, al-

* Received for publication, April 10, 1947.

though I have rarely observed it elevated above 10 units in the absence of hepatic or osseous disorder. The maximum normal level by the Bodansky method was accepted as 4 units.¹⁴

TABLE 1

SUMMARY OF CLINICAL AND LABORATORY FINDINGS IN 34 PATIENTS WITH INFECTIOUS MONONUCLEOSIS

CASE NUMBER	AGE	SEX	ICTERUS	EN-LARGED LIVER	WBC IN THOUSANDS	PER CENT LYMPHOCYTES	HETEROPHIL ANTIBODY TITER	ICTERUS INDEX	THYMOL TURBIDITY	CEPHALIN FLOCCULATION	SERUM PHOSPHATASE UNITS*
1	17	F	0	0	13.0	90	1:1792	8	—	4+	44 K.A.
2	23	M	0	0	17.0	77	1:224	—	—	3+	31 K.A.
3	25	M	0	0	6.7	44	1:7168	—	—	3+	8 K.A.
4	24	M	0	0	12.4	60	1:112	—	—	—	10 K.A.
5	23	M	0	0	10.0	79	1:1792	6	—	4+	14 K.A.
6	23	M	0	0	9.2	71	1:224	7	—	2+	16 K.A.
7	23	M	—	—	6.4	62	1:896	—	—	3+	9 K.A.
8	24	M	0	0	7.1	73	1:7168	—	—	3+	11 K.A.
9	24	F	0	±	6.8	69	1:7168	7	—	4+	36 K.A.
10	21	M	—	—	17.6	71	1:1792	—	—	4+	17 K.A.
11	21	M	0	0	12.0	61	1:896	—	—	4+	30 K.A.
12	20	M	0	0	9.8	56	1:1792	—	—	3+	12 K.A.
13	22	M	0	0	16.0	80	1:7168	5	—	—	11 K.A.
14	21	M	+	0	17.0	77	1:3500	17	19	4+	36 K.A.
15	20	F	0	0	16.5	78	1:1792	10	6	4+	16 K.A.
16	20	M	0	+	11.7	69	1:1792	9	15	4+	32 K.A.
17	28	F	±	0	9.0	60	1:7168	16	22	4+	14 K.A.
18	26	F	0	0	9.8	64	1:448	10	6	4+	10 K.A.
19	19	M	0	0	10.0	59	1:448	7	18	2+	12 K.A.
20	25	M	0	0	12.3	69	1:1792	6	29	3+	14 K.A.
21	22	M	±	0	12.0	66	1:7168	16	15	4+	13 K.A.
22	20	M	0	0	8.7	76	1:896	14	7	3+	15 K.A.
23	25	M	0	±	9.1	72	1:224	14	23	4+	20 K.A.
24	25	M	0	0	6.1	62	1:448	4	14	1+	14 K.A.
25	20	M	0	0	7.7	71	1:112	5	7	4+	9 K.A.
26	22	M	0	0	10.8	64	1:112	5	3	0	12 K.A.
27	29	M	0	0	7.6	74	1:224	7	10	3+	11 K.A.
28	15	F	0	0	11.0	71	1:3584	—	15	—	15 K.A.
29	35	M	0	0	16.8	83	1:448	—	—	—	12 Bod.
30	36	F	0	0	14.2	77	1:16	—	—	—	20 Bod.
31	28	F	—	—	8.5	75	1:2048	—	—	—	10 Bod.
32	30	F	—	—	14.8	55	1:1024	—	—	—	3 Bod.
33	20	F	—	—	5.3	54	1:1024	—	—	—	9 Bod.
34	22	F	0	0	11.2	74	1:3584	9	—	4+	12 Bod.

* K.A., King-Armstrong method; Bod., Bodansky method.

In 26 of the patients, the cephalin flocculation procedure of Hanger¹⁰ was employed and only a 3 or 4 plus flocculation was regarded as positive. In 20 instances, the icterus index was determined (normal 4 to 6 units),¹⁹ and in 15, the thymol turbidity test was performed (normal 1 to 4 units).¹⁵

RESULTS

The results obtained are tabulated in Table 1.

Serum phosphatase levels attained widely varied heights, the range being between normal and 44 King-Armstrong units and between normal and 20 Bodansky units. In only 5 patients were the readings within normal limits, while in 7 others, the increase was found to be considerable (over 30 King-Armstrong units).

Twenty-two of 26 patients showed a positive cephalin flocculation test and 3 of the remainder had 1 plus to 2 plus readings. Thymol turbidity readings were significantly elevated in all but one of 15 patients. The range of readings here was 3 to 29 units, the mean being 15 units.

Despite the detection of mild or equivocal icterus in 3 patients, 9 of 20 patients showed icterus index levels above 8. The range was narrow: 3 patients exceeded 15 units and the maximum level attained was 17 units.

At the time the initial blood samples were drawn, all of the patients were in the florid stage of the disease, although the duration of their illnesses varied between three and twenty-two days. Circumstances prevented serial blood studies in most of the patients, but in five instances the heterophil antibody titer, the serum phosphatase and the cephalin flocculation were each determined at weekly intervals for three weeks succeeding the initial observation. The heterophil antibody titer dropped progressively to negative levels in two patients, but in one patient, the titer remained 1:224 throughout the period of observation. The cephalin flocculation test was negative in the five subjects at the end of three weeks, as was the serum phosphatase. The latter levels dropped particularly rapidly, being normal in 3 patients one week after the original reading. Blood smears made at the end of the period showed essentially normal differential formulas with fewer than 30 per cent lymphocytes, but abnormal forms remained in small numbers.

In 28 patients, two or more tests were carried out (Table 1). In 18 of these patients, all the tests performed showed abnormal results, while in the remaining 10 subjects, at least one of the tests was abnormal. No constant relationship among the results obtained by these procedures was observed. In the 6 persons in whom only the alkaline phosphatase was determined, two showed normal values. Thus, evidence is at hand to point to some degree of interference with liver function in at least 32 of the 34 patients studied.

DISCUSSION

Both clinical and pathologic data indicate a high incidence of hepatic disorder in association with infectious mononucleosis, an incidence which cannot be determined on the basis of clinical jaundice alone. I believe that had additional tests been carried out in each of the subjects studied, all would have shown some alteration in liver function. This was so in the 15 cases reported by Cohn and Lidman.⁶

The basis for the elevation in serum alkaline phosphatase is not entirely clear.^{8,17,22} Although it is generally accepted that increase in this enzyme reflects

disorder of the liver or osseous system,¹⁷ Umenow²³ and others^{1,11} have reported such elevations in leukemia. Woodward and Craver,²⁶ however, found such increases to be rare in leukemia, but noted elevations frequently in the "lymphomatoid" diseases. These authors noted the presence of phosphatase in white blood cells, but felt that there was little likelihood that this contributed to the serum hyperphosphatasemia. There is the probability, in view of the common involvement of liver and bone by both leukemia and malignant lymphoma, that this invasion rather than the actual liberation of enzyme by leukocytes enhances the rise of serum phosphatase.

Hyperphosphatasemia in cases of obstructive jaundice was first reported by Roberts¹⁸ and was for some time regarded as a means of distinguishing obstructive from hepatocellular jaundice. Subsequent investigations, however,^{8, 20, 21, 22} have shown that this means of distinction is not valid and considerable variation may be anticipated. Certainly there is no indication whatever of the occurrence of significant biliary obstruction in the cases reported in this paper.

In view of the evidence of anatomic lesions in the liver in the occasional case of infectious mononucleosis available for such study and the striking frequency of abnormality evident in tests such as serum alkaline phosphatase, cephalin flocculation and thymol turbidity, carried out in 34 cases, it seems reasonable to conclude that liver involvement is of usual, rather than of incidental, occurrence in infectious mononucleosis.

SUMMARY

Thirty-four patients with infectious mononucleosis were studied to determine their serum alkaline phosphatase levels. In addition, the cephalin flocculation test was taken on 26 of the patients; the thymol turbidity test, on 15 patients; and the icterus index, on 20 patients. Significantly elevated alkaline phosphatase, slightly increased icterus indices and positive cephalin flocculation and thymol turbidity tests were found in these patients, whether or not clinical jaundice was present. Evidence is adduced to show that alteration in liver function as manifested by these tests is present in almost all patients with this disease.

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MENINGITIS CAUSED BY FRIEDLÄNDER'S BACILLUS OR *AEROBACTER AEROGENES*

REPORT OF TWO CASES WITH AUTOPSIES*

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Meningitis caused by Friedländer's bacillus is an uncommon clinical finding. In 1943, Ransmeier and Major⁵ collected 29 cases from the world literature and described one of their own. Since then, one case has been reported by King⁴ and one by Tartakoff, Grynbaum and Le Compte.⁷

The identification of the organisms in these cases raises once more the perplexing problem of differentiating between Friedländer's bacillus (*Klebsiella pneumoniae*, *Bacillus mucosus capsulatus*) and *Aerobacter aerogenes* (*Bacillus lactis aerogenes*, *Bacillus aerogenes*). Ransmeier and Major⁵ included in their series cases attributed to *A. aerogenes* because they are "closely related, if not indistinguishable" from Friedländer's bacillus. Schaub and Foley⁶ in their *Methods of Diagnostic Bacteriology* stated that infection with Friedländer's bacillus comprises a clinical rather than a bacteriologic entity. A heavily encapsulated, gram-negative bacillus isolated from the sputum of a pneumonia patient is usually regarded as a Friedländer's bacillus. The same organism isolated from a urine culture might be classified as *A. aerogenes*. In other words, designation of an organism as Friedländer's bacillus depends, primarily, on the source of the culture. According to this method of classification, the organisms in the cases to be discussed would be regarded as *A. aerogenes*.

Extensive studies and investigation of the cultural, biochemical and serologic characteristics of these two groups of encapsulated organisms, however, have failed to reveal any reliable criteria for their differentiation. Edwards² found that such tests as production of ammonia, reduction of nitrates, formation of indole, growth in uric acid and citrate mediums, and the methyl red and Voges-Proskauer tests are of no value in separating these two groups of gram-negative bacilli. The coagulation of milk, once looked on as an important diagnostic criterion, has given variable results in the hands of different investigators, even when they were working with the same strain of organism. The same holds true for the production of acid and gas from lactose. Clear-cut immunologic relationships between Friedländer's bacillus, Type B, and *A. aerogenes* have been demonstrated by Julianelle.³ The conclusion is that there are no constant differences between various strains of Friedländer's bacillus and *A. aerogenes*.

Consequently, we feel that designation of the organism as either *K. pneumoniae* or *A. aerogenes* is justified. However, we have chosen to label it *K. pneumoniae* (Friedländer's bacillus) in order to conform with the majority of the reports in the literature.

* Received for publication, April 9, 1947.

Recently, we had the opportunity to observe a patient who entered the hospital with a clinical picture of meningitis as a result of criminal abortion. Friedländer's bacillus was cultured from the blood and spinal fluid during life; at autopsy, the same organism was isolated from the blood, cerebrospinal fluid and uterus. Search of our files also has disclosed a case of meningitis caused by *A. aerogenes* in an 8 week old male infant with a congenital anomaly of the prostatic urethra, hydroureter and hydronephrosis. Culture of his cerebrospinal fluid yielded *A. aerogenes*. He recovered from this infection only to die of a subsequent *Escherichia coli* septicemia.

REPORT OF CASES

Case 1

Clinical data. A 29-year old white female, single, was admitted to McCook Memorial Hospital on December 5, 1946, and died December 12. She was five months pregnant and had had a criminal abortion performed by instrumentation of the cervix on November 30. On December 5, because of a persistent bloody vaginal discharge and fever, she consulted a physician, who referred her immediately to the hospital. On admission, her temperature was 100.6 F., pulse rate 112, respiratory rate 28 and her blood pressure 118/78. She was semicomatose and unable to extend her legs completely. The throat and pharynx were injected. The lungs and heart were normal. The abdomen was moderately distended, tender and tympanitic with some spasm. Pelvic examination disclosed a bloody discharge issuing from a soft and patulous cervix. The uterus was enlarged to the size of a five month pregnancy and was moderately tender. The neck was stiff, but no other abnormal neurologic signs were present. Lumbar puncture revealed cloudy, yellowish, fecal-smelling fluid under increased pressure. Forty cc. of cerebrospinal fluid was removed and 20,000 units of penicillin and 50 mg. of streptomycin were instilled. Culture of the fluid showed Friedländer's bacillus. The following day another lumbar puncture showed the spinal fluid unchanged except for the presence of some blood. The temperature ranged from 98 to 101.8 F. and the pulse rate continued to increase. On December 7, she developed convulsions and signs suggestive of tetany. The bronchi and trachea were filled with fluid and mucus. During aspiration of this material the respirations suddenly ceased and the patient died. Treatment had included a total of 950 mg. of streptomycin and 1,000,000 units of penicillin intramuscularly except for the initial intrathecal dose. In addition, she had had 7.5 gm. of sodium sulfadiazine intravenously and a transfusion of 500 cc. of blood.

Laboratory data. Hemoglobin 70 per cent; erythrocyte count, 3,100,000; leukocyte count, 11,000 to 28,500; NPN, 158 mg.; creatinine, 7.4 mg.; CO₂ combining power 26 volumes per cent; cerebrospinal fluid protein 605 mg., sugar 19 mg. Culture of the blood and of the cerebrospinal fluid each showed a pure growth of Friedländer's bacillus. A sample of the blood submitted to the Connecticut State Department of Health Laboratory was reported as containing *K. pneumoniae*, Type B. The organism was described as a gram-negative rod which, on MacConkey's agar, grew as convex colonies 3 mm. in diameter. The organism produced acid and gas from glucose, lactose, sucrose, salicin, maltose, mannitol, rhamnose, arabinose, raffinose, xylose, sorbitol and inositol. Dulcitol was unchanged. Growth on Kligler's iron agar produced acid and gas in the butt, an acid slant and no hydrogen sulfide. The organism grew on citrate and tartrate mediums. Purple milk and urea were unchanged. The Voges-Proskauer test was reported as plus-minus (\pm). Trimethylamine was produced but indole was not. The organism showed capsular swelling with *K. pneumoniae*, Type B antiserum.

Postmortem examination was done one and one-half hours after death. There were multiple excoriations about the mouth. The breasts were enlarged and yielded a watery colostrum on pressure. A copious, foul-smelling, bloody discharge issued from the vagina.

The spleen was of a soft mushy consistency and was enlarged, weighing 450 gm. The cortex of each kidney appeared swollen and edematous and scattered through the cortex were multiple minute yellowish spots, apparently representing early abscesses. The uterus was enlarged, measured 18 x 8 x 5 cm. and was of flabby consistency. There was a small amount of purulent exudate over the serosal surface at the attachment of the right tube. On opening the uterus, the cavity was seen to contain blood, pus and fibrin. There was a hemorrhagic mass about 3 cm. in diameter situated on the posterior aspect of the endometrial cavity near the fundus of the uterus. A thin seminecrotic membrane lined a portion of the uterine cavity. The cervix was hypertrophied and the external os was gaping. No lacerations were visible on the vaginal portion of the cervix. Surrounding the cervical canal, however, there was a thick cuff of recent interstitial hemorrhage averaging 2 cm. in thickness. The tubes showed a slight inflammatory exudate, as mentioned above. The right ovary contained a corpus luteum of pregnancy 1 cm. in diameter. The cerebrospinal fluid was cloudy. The brain weighed 1350 gm. and there was a copious yellowish exudate, which was most prevalent over the cerebral hemispheres. Relatively little exudate was present about the base of the brain. Multiple sections through the brain showed no evidence of inflammatory reaction within the brain substance. The lining of the fourth ventricle showed a few petechial hemorrhages. Cultures of the blood, meninges and uterus were taken.

Microscopically, the mass in the fundus of the uterus showed hemorrhage and necrosis of decidua extending into the uterine wall. A few trophoblastic cells were present. Section of the thin membrane that partly lined the uterus showed a largely necrotic decidual tissue with an extensive acute inflammatory reaction. Section from the region of the right tube showed an early acute inflammation. Section of the cervical canal showed an area of ulceration with a defect in the mucous membrane lining the cervix. Beneath this there was an extensive acute inflammatory reaction and a massive recent interstitial hemorrhage. The ovary showed a corpus luteum. Brain sections revealed an acute inflammatory exudate in the meninges. A section through the fourth ventricle disclosed petechial hemorrhages. The liver and spleen showed acute congestion and the kidneys displayed early minute abscesses. Cultures of the blood, uterus and meninges all yielded a pure growth of *K. pneumoniae* (*A. aerogenes*?). The anatomic diagnoses were: infected abortion (*K. pneumoniae*), septicemia (*K. pneumoniae*), acute purulent meningitis (*K. pneumoniae*), ulceration of endocervix with inflammatory reaction and interstitial hemorrhage.

Case 2

Clinical data. A male infant born at the Hartford Hospital, July 6, 1945, was readmitted on August 3, and died on September 2. The infant weighed 9 pounds 7½ ounces at birth and was normal during his neonatal stay in the hospital, leaving on his eighth day. At home he was a poor feeder on a formula of dextri-maltose. Two days prior to his readmission, his mother noted the passage of green watery stools. In addition, the child vomited brownish mucoid material. His urine was said to have been "reddish" on one occasion.

Physical examination on readmission showed a thin dehydrated male infant weighing 8 pounds 4 ounces. The temperature was 101.4 F. Aside from an injected pharynx, there were no remarkable findings. He was treated with hypodermoclyses and sodium sulfadiazine. Despite these measures, his temperature climbed to 103.6 F. Penicillin was started in a dose of 10,000 units every three hours. Three days after admission he developed convulsions and complete anuria. There was bulging of the anterior fontanel and moderate internal strabismus of the left eye. The nonprotein nitrogen at this time was 137 mg. Blood culture showed a heavy growth (4 plus) of *A. aerogenes*. Culture of the urine showed a heavy growth (4 plus) and of the cerebrospinal fluid a slight growth (1 plus) of the same organism. Spinal fluid protein was 122 mg. Penicillin was stopped and the child was given sulfathiazole. The nonprotein nitrogen rose to 198 mg. Cystoscopy was performed and moderate trabeculation of the bladder with intrusion of the lateral lobes of the prostate was found. An indwelling catheter was inserted, and within one week the nonprotein nitrogen

had fallen to 67 mg. The spinal fluid, however, still showed a 1 plus and the urine and blood a 2 plus growth of *A. aerogenes*. Treatment consisted of sulfathiazole supplemented with transfusions and hypodermoclyses. A third lumbar puncture, two and one-half weeks after admission, showed 36 cells of which 29 were polymorphonuclear leukocytes and 7 lymphocytes. Pandy's test was negative. At this time, cultures of the cerebrospinal fluid and blood were sterile. The child showed well marked improvement, took his feedings well, and had no diarrhea. Soon thereafter, he started to regurgitate his feedings. A barium enema and a gastro-intestinal series were negative. He vomited everything given by mouth and his condition progressively declined. A culture of the urine now showed a 4 plus growth of *E. coli*. Streptomycin was instituted but the child succumbed shortly.

Postmortem examination, done by Dr. Robert Tennant, revealed a congenital valve of the prostatic urethra with obstruction and a secondary bilateral hydronephrosis and pyelonephritis. There was terminal pneumonia and culture of the heart's blood revealed *E. coli*. The brain showed no evidence of meningitis.

SUMMARY

Two cases of meningitis are presented in which *Klebsiella pneumoniae* (Friedländer's bacillus) was found. Case 1 represents an instance of generalized infection with the organism following intra-uterine instrumentation. The only other case ascribed to a possible uterine focus is that reported by Davis and Fernando.¹ Case 2 is an example of apparent recovery from a generalized infection starting from the urinary tract. Meningitis associated with Friedländer's bacillus was uniformly fatal up to the advent of the antibiotics. With the use of these agents recoveries have appeared in the literature with increasing frequency.

These cases emphasize once more the difficulty, or impracticability, of attempting to differentiate between *K. pneumoniae* (Friedländer's bacillus) and *Aerobacter aerogenes* (*Bacillus lactis aerogenes*).

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CLINICOPATHOLOGIC CONFERENCE*

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Clinical data. The patient, a white male, 38 years of age, was admitted July 16, 1946, with complaints of weakness, nausea and vomiting, diarrhea, jaundice and pain and swelling of the abdomen, which had developed in the order named during the past two months. There had been occasional abdominal cramps. Because of the nausea, the patient had been able to ingest only small amounts of milk during the past month. Diarrhea had been constant and accompanied by much mucus and some bright red blood. Jaundice and abdominal swelling were painless and progressive. The patient recalled having had three or four similar episodes, the first in 1928. He had been a chronic alcoholic for about twenty years. While in the West Indies from 1924 to 1928, he had had malaria.

The patient was well developed, poorly nourished, lethargic, tremulous and apparently in acute distress. He had a foul, faintly alcoholic breath and his bed clothes were soiled by blood and feces. His skin showed deep yellow jaundice and over the shoulders and chest there were petechiae and "spider nevi". The pupils were normal and the fundi were not visualized. The lips and tongue were dry and the teeth carious. The lungs were negative except for moist râles in the right axilla. Blood pressure was 70/38. The heart was not enlarged, the rate was 80 and the rhythm regular. The abdomen was greatly distended and there was a fluid wave and shifting dullness. The liver edge was palpated 5 fingerbreadths below the costal margin. Periumbilical veins were prominent and retrograde flow could be demonstrated. There were no pathologic tendon reflexes. The prostate was not enlarged. There was slight pitting edema of the feet and definite tenderness of the calves on deep pressure.

The patient was given general supportive treatment, including a blood transfusion and glucose intravenously, but his condition rapidly became worse and he expired on the third hospital day.

Laboratory findings, July 17. Urinalysis: sugar 2 plus, albumin slight trace, bile 1 plus; Hb. 8 gm., erythrocytes 2,450,000, leukocytes 23,300, neutrophils 95, lymphocytes 4, monocytes 1 (neutrophils showed Döhle's inclusion bodies and marked toxic alteration); platelets 41, 650 per cu. mm.; blood urea 372 mg.; blood Kline test negative; albumin 1.2 gm., globulin 1.8 gm.; stools, occult blood 2 plus; icteric index 50; van den Bergh test direct, immediate, strong.

CLINICAL DISCUSSION

Dr. Robert J. Schneck. "The diagnosis must explain an acute illness of two months' duration with possible similar previous episodes, obstruction to the

* Received for publication, April 26, 1947.

portal circulation, hepatic enlargement, azotemia, anemia and leukocytosis. Cardiac decompensation *per se*, or congestive failure secondary to nephritis with liver enlargement and ascites, can be excluded because of absence of other signs of congestive failure. Neither primary nor metastatic carcinoma of the liver will explain all the findings. Amebic abscess of the liver is strongly suggested by his residence in the West Indies, leukocytosis and the abdominal findings. It is unlikely, however, that he could have had such an abscess from 1928 and the nitrogen retention is not easily explained on that basis. Weil's disease must be considered, but the absence of an acute severe illness preceding the jaundice makes this diagnosis untenable. Poisoning from some exogenous toxin must be seriously considered. During the prohibition era, we had several patients in this locality with liver enlargement, ascites and renal insufficiency which developed from consumption of an intoxicant containing an unidentified poison. Anemia and leukocytosis, however, were not part of the picture. This patient presented the findings which we commonly attribute to hepatic cirrhosis, including enlargement of the liver, changes in the serum proteins, edema of the ankles, ascites and spider nevi. His downhill course, however, was more rapid than is usually the case in uncomplicated cirrhosis. This fact makes me feel that, in addition to cirrhosis, there was a complicating acute hepatitis and that the rapid liver degeneration resulted in renal tubular damage and produced the so-called hepatorenal syndrome."

Dr. Richard M. McKean. "As I read the case, my attention was caught by the same factors that intrigued Dr. Schneck. This man was about 18 or 19 years old when he had his first attack, and it is this which concerns me most. We can explain his story on the basis of a common, garden-variety of cirrhosis with an acute episode at this point, but the patient recalled three or four similar episodes since 1928, or since he was 18 or 19 years old. This is difficult to explain. The juvenile cirrhosis, with spider nevi and petechiae, is usually, in my experience, not evanescent. Hypertrophic biliary cirrhosis has periods of recrudescence; acute episodes come and go, and each attack is associated with jaundice, diarrhea, abdominal pain and with enlargement of the liver, all of which were reported in this individual. If there is a pure biliary cirrhosis it is not, except in its terminal stages, associated with any degree of ascites. This man, however, was without adequate food for at least two months. He had a hypoproteinemia with an inverted A/G ratio which might account for the accumulation of fluid in the abdomen. However, an ascites with inversion of A/G ratio should be associated with fluid elsewhere and, as I note, there was only slight pitting edema of the feet. That is rather unusual if we are to ascribe the anasarca to lowered blood protein and an inversion of the A/G ratio. I think that intrinsic liver disease best explains this case. This boy had the reputation of being an alcoholic from the age of 16. He had been in the West Indies from the time he was a youth, which leads me to suspect that he may have had some tropical disease. Amebiasis best fits this picture, and could account for the nausea, vomiting, abdominal cramps and weakness, and with an invasion of the liver through the portal circulation might very well have

produced; as not uncommonly happens, a diffuse hepatitis followed by a localized abscess formation. While the recurrent nature of the illness cannot be explained on that basis, the terminal picture might be. I should be inclined to hypertrophic biliary cirrhosis as my diagnosis of choice, although I admit that it certainly would be unusual to see it over a period of almost twenty years."

Dr. Charles G. Johnston. "As I read this conference sheet, two possibilities struck me. Either this is a case of amebiasis contracted in the West Indies, or a very good case of portal cirrhosis. I believe that amebic abscess probably fits the picture better than cirrhosis. One must not forget, however, that the patient came into the hospital in a terminal state. When an individual is hospitalized in such a condition, it is usually difficult to get his story and many of his physical findings may be altered by dehydration. One might explain his high urea nitrogen on the basis of dehydration, but not the low platelet count. The 2 plus sugar in the urine may be similarly explained, and Dr. Brines pointed out before we began that the patient excreted practically no urine. It is surprising that a man with such severe dehydration as is here suggested should have only a 2 plus albumin. It is perfectly possible to relate practically all of this man's symptoms, except the jaundice, to the high blood urea and marked nephritis. Now there are two factors that I should like to bring out. One is that this man had ascites and an enlarged liver; there is nothing to indicate that the ascites which he had was a simple ascites. No tap was made, and so it is perfectly possible that he may have had bile ascites from an obstructive jaundice with perforation of the gallbladder or common duct. He was surely sick enough to have had a biliary ascites. I also call your attention to the fact that his blood pressure was down to 70/38, yet his heart rate was only 80. In jaundiced patients and, frequently in those who have biliary ascites or rupture of a bile passage, it is quite common to find the pulse rate low. Now for some reason, it appears that in the anamnesis the temperature has been carefully omitted. I would suspect that this man, if he wasn't too far gone, had a fever in keeping with a leukocytosis of 23,300, so that from a surgical standpoint, this man probably had acute appendicitis two months before July 16, with an extension of the infection into the portal system, a pylephlebitis, followed by thrombosis of the portal vessels and ascites. Of course, the portal vein may be obstructed from many causes. I have seen obstructive jaundice occur in a patient following rupture of a duodenal ulcer, by formation of a large subhepatic abscess and pressure on the ductal system and portal vein. It is my feeling that this man had an intraperitoneal infection which began either as an acute appendicitis or as a subhepatic abscess. In either event there was extension to the liver, or obstruction of the ducts and portal vein, with resulting jaundice and ascites."

Dr. Ralph A. Johnson. "I would underscore one point very strongly, and that is that carcinoma of the liver occurs in a pre-existing cirrhosis much more commonly than in a healthy liver. The pattern here is quite clearly that of portal cirrhosis. I am always suspicious of a protocol in which all the roads lead to one diagnosis. It is generally a trick to catch the unsuspecting clinician. Nevertheless, I would make my first diagnosis portal cirrhosis with secondary car-

cinoma of the liver and carcinomatosis. Among the other possibilities are Hodgkin's disease and leukemias. I can't see how a high blood urea with the mild degree of nephritis that this patient was supposed to have had, can be ignored and I would postulate a hypernephroma to explain it in part."

Dr. A. Hazen Price. "It is possible that we have here a portal cirrhosis of the Laennec type, a hypertrophic biliary type, or a mixed type of cirrhosis, because the liver was large. Most patients with portal cirrhosis of the Laennec type have a comparatively small liver, while patients with a biliary cirrhosis commonly have a large liver. Since this condition started early in life, the patient could well have had a biliary type of cirrhosis. I think, however, that we ought to consider another possibility that has not been mentioned, and that is that this patient had recurring attacks of pancreatitis. Recurring attacks of acute pancreatitis occur not infrequently over a period of years, with subsequent liver damage. The classical picture of acute hemorrhagic pancreatitis with acute epigastric pain, boardlike rigidity, a high white count, moderate cyanosis, together with shock is not difficult to recognize, particularly when there are facilities to do amylase determinations. But the possibility of chronic recurring attacks of pancreatitis is not considered often enough. Dr. Johnson mentioned one other possibility, Hodgkin's disease, and I think it should be particularly emphasized because this patient's trouble started when he was a boy, and that is the time that Hodgkin's disease is very common. Hodgkin's disease not infrequently masks itself by limiting its involvement entirely to the viscera or to the nodes about the liver without affecting the peripheral or mediastinal nodes. It commonly produces a bizarre picture such as this individual presented. Again, if we had known that he had recurring types of elevations of temperature like we see so frequently in Hodgkin's disease, it would have been of help, but the patient was in the hospital for only three days. The high white blood cell count with a high percentage of neutrophils is common in Hodgkin's disease. It would be interesting to know if the neutrophils were predominantly of mature type as they frequently are in Hodgkin's disease. I would like to suggest Hodgkin's disease and chronic recurring pancreatitis as possible diagnoses in this case."

Dr. Paul H. Noth. "One thing is central in both physical and laboratory findings, and that is that this patient had severe chronic diffuse liver damage, resulting in a picture chiefly of hepatic coma. No diagnosis other than that would explain the findings. I don't believe that obstructive jaundice *per se*, carcinomatous involvement of the liver alone, or amebic abscess as sole diagnoses could explain such severe hepatic insufficiency. The type of cirrhosis is largely speculative, both types mentioned, atrophic and hypertrophic primary biliary cirrhosis, being possibilities. This man did not have a history of obstructive biliary cirrhosis secondary to biliary tract disease. Frequently, we fail to realize that there are a great many cases of chronic hepatitis which don't fit into any of these groups. One cause of chronic hepatitis is catarrhal jaundice or non-specific infectious hepatitis. Knowing Dr. Brines' interest in carcinoma, I would consider the superimposition of carcinoma on cirrhosis. The consistency

of the liver and the presence or absence of nodularities, and the type of ascitic fluid present are not described. If the ascitic fluid were bloody, it might well indicate carcinoma. Considering these omissions, carcinoma is a very likely complication, although entirely speculative. Another chronic condition which should be mentioned, suggested by the history dating back to early adulthood, but not borne out by the findings, is chronic hemolytic icterus. Since it would have to be complicated by obstructive jaundice and hepatitis, I don't think that it is a good explanation. Jaundice in a patient with cirrhosis is ordinarily thought of as a very severe complication, one that is likely to be terminal, but we see many patients who have underlying cirrhosis with recurrent episodes of acute hepatitis of varying severity. Many such patients live for years after their initial attacks of jaundice. I think that this is the explanation of the recurrent jaundice in this case. Another thing commonly seen at autopsy to explain the rather frequent finding of a large liver associated with portal cirrhosis, is lipoidosis of the liver. I think that this patient definitely had underlying chronic hepatitis of some sort. There was probably also an acute episode of hepatic degeneration, associated lipoidosis, and possibly complicating primary carcinoma of the liver."

Dr. David I. Sugar. "No one has commented on the petechiae. These are evidence of septicopyemia. Here was a man with a big liver and spleen and leukocytosis, who had not only occult blood in his stool, but also blood in his bed. One of the causes of high nonprotein nitrogen is hemorrhage into the intestinal tract. The individual was an alcoholic. I think that his liver was rapidly enlarging, due to obstruction of the portal vein and that the portal vein obstruction was due to malignancy with secondary infection, which was seeding the blood stream, giving him septicemia. I just want to make one diagnosis, Chiari's disease, obstruction of the portal vein. This was produced by a new growth and complicated by secondary infection and septicemia."

HEMATOLOGIC INTERPRETATION

Dr. Lawrence Berman. "Three parts of the hemogram of this patient require our attention: anemia, neutrophilia and thrombocytopenia. The patient had a macrocytic normochromic anemia of 2,450,000 erythrocytes per cu. mm. We must consider the temporary macrocytosis associated with a high reticulocyte count after blood loss, pernicious anemia and other macrocytic anemias. The absence of frank massive hemorrhage rules out post-hemorrhagic anemia and the leukocytosis is not typical of pernicious anemia. Among the diagnostic possibilities suggested by the clinicians, cirrhosis of the liver, in which macrocytosis is common, remains important. The leukocyte count of over 24,000 cells per cu. mm., the marked right shift, and the presence of Döhle's bodies point to severe toxemia or infection which can best be explained on the basis of an acute process. The low platelet count of 41,000 per cu. mm. may be a feature of myelophthisic anemia, Werlhof's disease, toxemia of animal, vegetable, or chemical origin, or of hematopoietic diseases, such as leukemia. I think the clinical features of the case rule out these possibilities, with the exception of

Hodgkin's disease which was emphasized by Dr. Price. However, if Hodgkin's disease were to explain the severe chronic anemia in this patient, we would expect an anemia of somewhat different type, namely a myelophthisic anemia, in which the erythrocytes are likely to be normocytic and normochromic, with nucleated red cells in the blood. Furthermore, when there is an associated leukocytosis with myelophthisic anemia, we expect to find a left shift or leukemoid pattern in the blood, and this was absent. If we must choose from among the diagnostic suggestions already made, cirrhosis of the liver with a complicating infection or toxemia seems to be the best explanation of the blood findings."

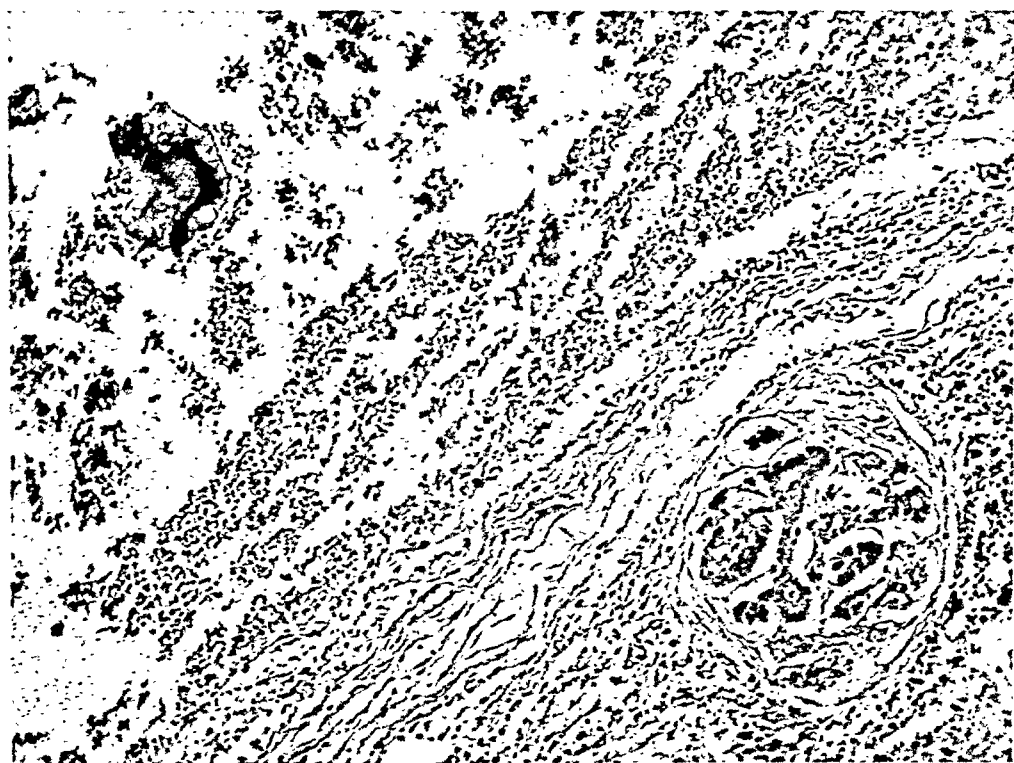


FIG. 1. Section from wall of a pancreatic abscess. Note microcalculus in upper left hand corner. Hematoxylin and eosin. $\times 105$.

PATHOLOGIC REPORT

Dr. Bert E. Stofer. "The chief pathologic findings were in the pancreas. This organ was almost completely replaced by multiple abscesses. Figure 1 represents a section from the wall of one of the pancreatic abscesses. The abscesses contained microcalculi. There was increase of fibrous tissue in the pancreas, both within and about the lobules. The inflammatory process extended to the tissues surrounding the duct and had occluded it by pressure from without. Extension of the abscess to the colon had resulted in a small perforation near the hepatic flexure, and it was from this perforation that the bleeding from the intestine was thought to have arisen. The kidneys were enlarged, weighing 300 gm. each, and the medullary portions were bile stained. The liver was the site of an active portal cirrhosis. Its great enlargement was due to massive lipoidosis.

The microscopic study, together with the gross appearance of the organ, led us to the conclusion that the disease was essentially that of a terminal, acute, suppurative exacerbation of a chronic pancreatitis, an entity which has recently been described under the term 'chronic relapsing pancreatitis'."

CLINICOPATHOLOGIC CORRELATION

Dr. Osborne A. Brines. "Considering that the liver in this case weighed 5 Kg., one of three types of cirrhosis would have to be considered: portal cirrhosis, obstructive biliary cirrhosis and primary hypertrophic biliary cirrhosis (Hanot). Obstructive biliary cirrhosis can be ruled out on a histopathologic basis in the absence of chronic biliary obstruction. Hanot's hypertrophic biliary cirrhosis probably does not need to be seriously considered as an entity because we believe it to be a variety of portal cirrhosis. Portal cirrhosis with extreme fatty metamorphosis, called fatty cirrhosis by some, is the diagnosis we prefer to apply here. The cirrhosis appears to be independent of the pancreatic disease but the lipoidosis may have been due to a deficiency of a lipotropic factor, such as lipocaic (as proposed by Dragstedt, *Arch. Int. Med.*, **64**: 1017-1039, 1939), and therefore might be the result of pancreatic insufficiency. The recurring attacks of upper abdominal pain over a period of years and the histologic evidence of chronic interstitial pancreatitis suggest that this case might fall into the group of cases described by Comfort, Gambill and Baggenstoss (*Gastroenterol.*, **6**: 376-408, 1946) as chronic relapsing pancreatitis. As in their cases, there was no biliary tract disease and the terminal common duct obstruction and hydrops of the gallbladder could be secondary features. The suppuration, and certainly the perforation of the peripancreatic abscess into the colon, are unusual features. The azotemia in this case could probably be entirely prerenal on the basis of vomiting and diarrhea with resulting dehydration. However, each kidney weighed 300 gm. and there was acute degeneration of tubular epithelium and some interstitial edema. A diagnosis of nephrosis is obviously correct. To classify the nephrosis as cholemic is permissible, but perhaps inadequate. That there was circulatory collapse in this case was evidenced by the extremely low blood pressure which reminds one of the work of Brun and Knudsen and their co-workers at Copenhagen (*J. Clin. Investigation*, **25**: 568-574, 1946) in which unmeasurably low blood pressure values were produced by the passive erect posture and followed by post-syncope oliguria. In this case the output of urine was extremely low and there seems to be little doubt that the diminished glomerular blood flow and capillary blood pressure with the associated diminution in peritubular circulation resulted in anoxia and renal ischemia. As to whether the term "hepatorenal syndrome" might apply in this case, the answer can only be that the term is a vague pathologic concept which probably should not be employed when jaundice is present.

"The final diagnoses were chronic relapsing pancreatitis, portal cirrhosis and lipoidosis, and cholemic nephrosis."

EDITORIAL

THE FUNCTIONS OF THE CONNECTIVE TISSUE

In recent years, increasing attention has been devoted to the investigation of the specific physiologic functions of the connective tissue, factors which affect its activity, and chemical and pathologic changes which are found associated with it in various disease processes.

Besides its rôle as a supporting structure, the connective tissue has been described as a semipermeable barrier between the canalicular vessels and the parenchymal cells, as a depot for the storage of nutrient materials and as a regulator of the water and salt balance of the body. In addition, plastic and protective activities, such as healing of wounds, and antibody formation, have been considered as functions of this tissue.

Extracellular substances found in the space between the blood vessels and the parenchymal cells are believed to be products of mesenchymal cellular activity. Several of these substances have been isolated and their properties partly identified. The greater portion of the protein component of connective tissue has been found to consist of collagen; elastin and reticulum are also present. Chondroitin sulfuric acid and hyaluronic acid are the most extensively studied polysaccharides; other sulfuric acid esters of polysaccharides have been described by Dr. Karl Meyer and others.

Hyaluronic acid has been isolated in significant quantities from synovial fluid, aqueous and vitreous humors, Wharton's jelly and skin. Hyaluronic acid is also present in the capsules of certain streptococci, and is believed to be present in the jelly-like material surrounding the ovum as it is extruded from the ovary.

Hyaluronidase, which depolymerizes hyaluronic acid, occurs in the mammalian testis, eye and skin, many pathogenic bacteria, poisonous insects, leeches and snake venoms. This enzyme complex is believed to be present in an inactive form in the skin. Hyaluronidase is identical with the "spreading factor" of Duran-Reynals and can be demonstrated to increase the area of spread of intradermally injected particulate substances such as India ink, dyes, viruses and bacteria. This action is the result of depolymerization of the gel-like hyaluronic acid in the connective tissue.

Many factors have been reported to influence the composition and activity of the connective tissue. Among these, sex, age, hereditary background and hormonal influences have been listed. Also, the Russians claim that a serum known as anti-reticular cytotoxic serum, which they have prepared is capable of altering the composition and function of the connective tissue. Preliminary studies in this country and in England have not entirely fulfilled early therapeutic expectations of this serum.

In recent years, many interesting concepts have emerged from studies of mesenchymal diseases. Klemperer, Pollack and Baehr have emphasized that the connective tissue is the tissue primarily involved in the so-called diffuse collagen diseases. The latter include disseminated lupus erythematosus, scler-

roderma and rheumatic fever. These authors have pointed out that the response to certain noxious stimuli may be collagenous rather than cellular (*e.g.*, keloid formation).

Rich has postulated that anaphylactic hypersensitivity may be a factor in the development of the typical lesions of a variety of mesenchymal diseases. This view is supported by the work of Rössle and of Jaeger who pointed out that fibrinoid degeneration is a constant and characteristic feature of diseases of allergic or pathergic type. Selye and others consider that certain endocrine factors, especially those involving the pituitary-adrenocortical axis, may be important in the pathogenesis of certain connective tissue diseases. Selye has succeeded in the experimental production of Aschoff nodules, lesions typical of periarteritis nodosa and lesions suggestive of rheumatoid arthritis, by administration of large doses of desoxycorticosterone acetate.

These investigations would seem to deal with influences affecting a common biologic system. It is believed that study of the basic chemistry and physics of the connective tissue will further elucidate the behavior of this tissue in health and disease and in the correlation of available information. The observation of Watson and Pearce on pretibial myxedema, reported in the current issue of the Journal, is a step in this direction.

COORDINATION OF CANCER RESEARCH

In this issue of the Journal, Simpson, in a paper on "Progress in Cancer Research", briefly summarizes some of the principal present research on cancer. The author visited many cancer research centers in the United States where he studied the data with the investigators themselves. As a result of these visits, he has pointed out the need for correlation of the findings of all cancer investigators. The sponsor of this study was the Charles F. Kettering Foundation of Dayton, Ohio. For many years, Dr. Kettering has exhibited a lively interest in medical research in general and his present interest in the cancer problem should be welcomed by all workers in this field. He believes that if some of the modern methods of group engineering were to be applied to cancer research, the solution of the cancer problem might well be hastened.

It is generally recognized that there is too great a tendency for research workers to direct their interest entirely to their own particular sphere of the problem, to the exclusion of other related aspects. Cancer has so many highly complex facets that no single research worker or single group of investigators can possibly deal adequately with all of them. Concerted effort on the part of many groups of research workers on a world-wide scale should result in further significant advances in our knowledge of cancer.

Constant correlation and integration of the findings of all research groups in cancer through a central agency, such as the new World Health Organization of the United Nations, would certainly be a constructive development.

BOOK REVIEWS

A Laboratory Manual of Physiological Chemistry. Ed. 6. By D. WRIGHT WILSON, Benjamin Rush Professor of Physiological Chemistry, University of Pennsylvania. 275 pp., 3 tables. \$2.50. Baltimore: The Williams & Wilkins Company, 1947.

This laboratory manual, now in its sixth edition, was first published in 1928 and is designed for medical, dental and veterinary students of physiologic chemistry. While the manual is "arranged for students familiar with elementary inorganic, theoretical and organic chemistry", some of the introductory experiments preclude this premise. On the whole, the experimental material exceeds that which can be covered in most courses of physiologic chemistry.

The experiments on biologic fluids, such as saliva, gastric juice and milk, include all classic material. The quantitative work on blood is prefaced with good descriptions of and directions for both visual and photo-electric colorimeters. The quantitative blood methods described are quite inclusive. There are appropriate sections on the composition of tissues, such as bone and muscle. The experiments on bile might better have been included with the digestive fluids, rather than immediately preceding the section on urine. The division on metabolism might have been improved by the inclusion of specified diets, rather than by placing reliance on the student's choice and computation, in order that the general urinary picture of the effects of foodstuffs might be observed.

In general, the emphasis on physical chemistry (colloids, pH, spectroscopic examination of blood pigments) is much in evidence, yet a proper discourse on the salting out of proteins as a colloidal phenomenon is lacking. A rather large number of biochemical preparations is included although some are of doubtful value in elementary physiologic chemistry for medical students. The vitamin-deficiency experiments on animals serve to round out the volume. This laboratory manual is somewhat beyond the needs of most courses in physiologic chemistry in quantity, but not in quality.

Detroit

ARTHUR H. SMITH

Penicillin in Syphilis. By JOSEPH EARLE MOORE, M.D., Associate Professor of Medicine and Adjunct Professor of Public Health Administration, The Johns Hopkins University. 319 pp., 57 figs., 52 tables. \$5.00. Springfield, Illinois: Charles C Thomas, 1947.

The fact that penicillin is effective in the treatment of syphilis has been widely disseminated through both the lay and professional press. There has been an urgent need for an authoritative statement on its limitations and effectiveness in the various clinical types of syphilis, as well as recommended time-dose relationships. Dr. Moore is eminently qualified to make such an appraisal of penicillin in syphilis. As chairman of the Subcommittee on Venereal Diseases of the National Research Council and of the Syphilis Study Section of the National Institute of Health, he has been in charge of both the clinical and experimental investigations of penicillin in the treatment of syphilis. A controlled investigative program on such a large scale is unique in experimental medicine. Forty-four groups and clinics participated in this organized nation-wide study including 4 installations of the United States Army, 1 of the Navy, 13 United States Public Health Service Rapid Treatment Centers and 26 civilian clinics. In addition, 8 laboratories of experimental syphilis are cooperating.

This monograph represents the first authoritative and systematically presented statement on how best to employ penicillin in the several stages of syphilitic infection. It is recognized that frequent revision will be required to maintain it up-to-date. This monograph will supply the basic principles on which the evaluation of penicillin must be made. It will be up to the physician to keep informed of rapid future developments through the medical literature in periodicals. However, on the basis of an understanding of the sound analyses of both clinical and experimental work reported in this monograph, he will be in a better position to separate the "wheat from the chaff". This monograph consists of 19

chapters. The first 8 are devoted to fundamental information concerning the chemistry, pharmacology, therapeutic activity as determined through experimental animals, and the mechanism of action of penicillin. Penicillin cannot be used intelligently without a clear understanding of this fundamental information, not only in syphilis but in infectious diseases in general.

Dr. Moore emphasizes that this monograph is not intended to replace, but simply to supplement, his larger and earlier text, *The Modern Treatment of Syphilis*. The intelligent use of penicillin in syphilis depends on an understanding of the fundamental, underlying problems of the biology and prognosis of syphilitic infection itself.

The collected experiences in the use of penicillin clinically and the author's recommendations for dosage to be employed are discussed in Chapters 9 to 17. A chapter is devoted to the treatment of early syphilis, arsenic-resistant early syphilis, latent syphilis, benign late syphilis, cardiovascular, ocular, prenatal and congenital syphilis and neurosyphilis. The treatment of early syphilis and neurosyphilis is very thoroughly discussed. The concise summaries at the end of each of these clinical chapters are the most authoritative statements available to the physicians' questions on how to use penicillin most effectively in such cases. This book should be a "must have" for all physicians who treat syphilis.

Detroit

L. W. SHAFFER

Die Hormonversorgung des Foetus. By DR. JULES SAMUELS, Chirurg Frauenzart Spezial Arzt für endogene Endokrinotherapie, Amsterdam. 320 pp. 2 figs. Leiden: E. J. Brill, 1947.

The author presents a review of the world's literature, with 544 references, on gonadotropic substances and concludes that these hormones are vitally concerned with the regulation of fetal development. The maternal hypophysis is the only source of the gonadotropins during the first weeks of pregnancy. After the ovum has successfully embedded itself, this function is taken over by the chorionic villi. While gonadotropic hormone activates fetal cell divisions, the further growth of the fetus depends on the luteinizing factor. The latter is secreted exclusively by the pituitary eosinophilic cells which become hyperplastic while the number of the basophilic cells, which are the source of the follicle stimulating principle, diminishes. Samuels contends that the luteinizing factor is identical, not only with the growth hormone, but also with the thyrotropic hormone.

The book will be useful as a literary review on gonadotropins during pregnancy even though it is disappointing to those who from the title of the book had hoped for a clarification of a controversial subject by original chemical, experimental or clinical facts.

Wichita, Kansas

C. A. HELLWIG

Charles-Édouard Brown-Séquard. A Nineteenth Century Neurologist and Endocrinologist. By J. M. D. OLMSTED, M.A. (Oxon.), Ph.D., D.Sc., Professor of Physiology, University of California. 253 pp. \$3.00. Baltimore: The Johns Hopkins Press, 1946.

This biography of the famous nineteenth century investigator, Charles-Édouard Brown-Séquard, is a compilation of three papers given in the Hideyo Noguchi Lectures at Johns Hopkins University in February 1946. The lectures engagingly tell the story of this indefatigable worker. The account of his numerous travels, often taken in flight from seemingly intolerable conditions, and the record of his hopes, fears, ambitions, loves, jealousies and foibles are well told.

While his later work on himself in an attempt to regain youthful vitality by means of injections of testicular extract is well known, it is not so well known that this tendency to work on himself characterized his whole professional career. Early experiments on himself involving recovery of gastric juice with a sponge led to the habit of rumination which proved to be embarrassing throughout his life.

For his work on the nervous system, he will always be remembered; it is inconsequential that his work on epilepsy was unsound. It does not matter that he exaggerated a few favorite theories; it was part of the humanness of the man. Even though he died five years

after starting the testicular extract injections for which he claimed rejuvenating powers, he succeeded in focusing the attention of the medical world on the endocrines.

As teacher, research worker, physician and professor, he was known in England and America, as well as in France. As often as not, he failed to fulfill the expectations of those who attempted to woo him away from his original love, physiologic research. Finally, with the death of Claude Bernard in 1878, Charles-Édouard Brown-Séquard succeeded to the Chair of Medicine at the Collège de France, the most prominent position in French scientific circles. With the help of d'Arsonval, the aging scientist was able to carry out the research and give the lectures which his eminent position required, until his death on April 1, 1894. Even in his last illness, he could not forego the habit of making observations on himself, for he noted daily the progress of the vascular lesion to which he eventually succumbed.

Physiologists, medical students and physicians alike will enjoy this biography.

Detroit

L. J. BAILEY

X-ray Diffraction Studies in Biology and Medicine. By MONA SPIEGEL-ADOLF, M.D., Professor of Colloid Chemistry and Head of the Department of Colloid Chemistry; and George C. Henny, M.D., Professor of Medical Physics and Head of the Department of Physics, Temple University School of Medicine. 215 pp., 86 figs., 12 tables. \$5.50. New York: Grune and Stratton, 1947.

Perhaps the best index of the high character of this book is to be found in a consideration of the authors themselves. Dr. Spiegel-Adolf has turned out much original work of outstanding quality and Dr. Henny has long been known for the excellence of his researches, largely in the field of radiology. It would be difficult to choose a more suitable team to cover the borderline field where radiology overlaps chemistry and cytology. A third personality who is mentioned by the authors in their preface as having been of great assistance to them is Dr. W. Edward Chamberlain who has a special genius for helping others turn out excellent research.

The book is not too technical for the average physician to enjoy. It begins with an introduction which gives a brief but adequate history of the method and some general ideas as to its practical applications. This is followed by a brief outline of the theoretical aspects which, like many other parts of the book, are very cleverly abstracted. A beginner can get enough from this and the other technical chapters to have a working idea of the method and its underlying theoretical principles, without being burdened by a mass of exact theory which might cause him to give up with the feeling that the method is beyond him.

A person trained in diffraction work may be a bit startled to find that Chapter 3 entitled "Interpretation by X-ray Diffraction Patterns" is only nine pages long. However, this brief section carries enough of fundamental truth to permit the beginner to gain a satisfactory idea of a subject concerning which many volumes have been written.

There follow approximately 140 pages of direct application of the method and the techniques to specific substances, such as carbohydrates, proteins and lipid substances and specific structures which are of fundamental and direct importance in all of the biologic sciences, including medicine. Anyone familiar with the microscope and its limitations can easily learn how the diffraction method can be used to advantage to extend his observations beyond the range of the microscope.

One of the most important features of the book is the splendid bibliography which characteristically has been well chosen.

The binding, paper and format are of excellent quality. The type is clear and free of errors. The illustrations are well chosen and clearly reproduced.

The book offers an excellent beginning for anyone planning to enter the field, and an excellent survey for others, not engaged in this work, who wish to learn what is being done and what can be done by the diffraction method.

Detroit

K. E. CORRIGAN

NEWS AND NOTICES

TENNESSEE SOCIETY OF PATHOLOGISTS

The Tennessee State Society of Pathologists was formed April 9, 1947, at a meeting of the State Medical Association in Memphis. Fourteen pathologists attended the organizational meeting. The Society plans to hold meetings at least once a year with a program of scientific papers and post-graduate instruction. The following officers were elected: Dr. Alfred Golden, Memphis, president; Dr. W. A. DeMonbreun, Nashville, vice president; and Dr. T. C. Moss, Memphis, secretary-treasurer.

SOCIETY FOR THE STUDY OF ARTERIOSCLEROSIS

The organizational meeting of The American Society for the Study of Arteriosclerosis was held June 9, 1947, at 9:00 A. M., at the Hotel Strand, Atlantic City, New Jersey. The organizing committee was composed of four clinicians: Dr. G. R. Herrmann, Galveston; Dr. R. A. Katz, New Orleans; Dr. W. B. Kountz, St. Louis; and Dr. J. B. Wolfe, Philadelphia; and four experimentalists: Dr. G. L. Duff, Montreal; Dr. H. Goldblatt, Los Angeles; Dr. W. C. Hueper, New York; and Dr. I. H. Page, Cleveland. Clinicians and experimentalists interested in an intensified investigation of all phases of this disease are invited to join the organization. All inquiries should be addressed to Dr. O. J. Pollak, Wilmington General Hospital, Chestnut and Broom Streets, Wilmington 14, Delaware.

SOCIETY FOR THE STUDY OF BLOOD

The American Society for the Study of Blood held its organizational meeting June 8, 1947, at 1:30 P.M., in the Hotel Claridge, Atlantic City, New Jersey. The purposes of this organization are to further the study of blood and to disseminate knowledge concerning clinical hematology, blood grouping and blood and plasma transfusions. The membership is open to individuals who are actively engaged in this field or who devote a significant portion of their time to it.

CERTIFICATION OF DENTAL SPECIALISTS

The Council on Dental Education of the American Dental Association has announced the requirements for certification of dental specialists in the following five fields of dental practice: oral surgery, orthodontics (teeth-straightening), pedodontia (children's dentistry), periodontia (treatment of gum diseases) and prosthodontia (artificial dentures). Following the same general pattern used in the certification of medical specialists, the Council urged that the certification of dental specialists be national in scope and under the voluntary control of the professional groups involved. The American Board of Oral Surgery and the American Board of Orthodontics have already been established, while the remaining three specialties will establish boards in the near future.

PERSONALS

Dr. Paul A. Van Pernis, pathologist and director of laboratories at Butterworth Hospital, Grand Rapids, Michigan, was formerly a resident in pathology at St. Luke's Hospital, Chicago, and was not the pathologist at that hospital, as erroneously stated in the February issue of the Journal.

Dr. Everett L. Bishop has been promoted from Associate Professor of Pathology to Professor of Pathology (Neoplastic Diseases) at Emory University School of Medicine.

TECHNICAL SECTION

EVALUATION OF VOLUMETRIC DATA OBTAINED BY CENTRIFUGATION OF ASPIRATED STERNAL MARROW OF ADULTS

I. ESTIMATION OF RELATIVE FAT CONTENT*

LAWRENCE BERMAN, M.D., AND ARNOLD R. AXELROD, M.D.

*From the Departments of Pathology and Medicine, Wayne University College of
Medicine, and the Anemia Laboratory, Out-patient Department
Harper Hospital, Detroit*

The value of volumetric data obtained by centrifugation of aspirated sternal marrow by the method of Schleicher and Sharp⁷ has been pointed out by several authors.^{1, 2, 3, 6} The literature contains few references to data of actual observations in normal and pathologic conditions.^{4, 5, 6} The fat content of sternal marrow of adults varies in different clinical conditions (see Figs. 1 and 2). In order to determine the relative fat and cell content of aspirated sternal marrow, it was necessary for us to evaluate the volumetric data obtained by centrifugation of sternal marrow in adults. In this paper, we wish to state the results of a study of the relative fat content of such material.

MATERIAL AND METHODS

The aspirated sternal marrow material was analyzed from 62 patients. The diagnoses of these patients were as follows: cirrhosis of the liver, 17 cases; pernicious anemia, 10 cases; anemia of undetermined origin, 6 cases; carcinomatosis, 5 cases; normal individuals, 4 cases; chronic microcytic hypochromic anemia, 3 cases; leukemia, 2 cases; multiple myeloma, 2 cases; macrocytic nutritional anemia, 2 cases; purpura hemorrhagica, 2 cases; sickle cell anemia, lymphoblastoma, acute gastritis, tuberculosis, lobar pneumonia, agranulocytosis, ovalocytic anemia, aplastic anemia and hemochromatosis, 1 case each. The volumetric data were obtained as previously described.^{2, 3, 7} The sections of the particles of aspirated marrow were prepared by the methods described by Schleicher⁶ and modified by Berman and Axelrod.² The relative area occupied by fat in the sectioned material was estimated with the aid of the Whipple eyepiece micrometer,^{1, 6} as shown in Figure 3. No attempt to calculate the actual fat content of the samples was made, since the factors of shrinkage or distortion of tissue by fixation cannot be accounted for with absolute accuracy. The figures arrived at are relative estimates, not absolute values.

Since all of the particulate material which was obtained in each case was prepared for sectioning serially at a thickness of from 6 to 8 microns, error due to sampling was eliminated. Nevertheless, a study was made to determine what differences, if any, in the estimates of fat area in the sectioned material occurred as a result of averaging 10, 50, 100, or 200 consecutive estimates of fat content

* Received for publication, April 22, 1947.

TABLE 1

COMPARISON OF ESTIMATES OF FAT CONTENT BASED ON A STUDY OF THE FIRST AND SECOND CONSECUTIVE GROUPS OF FIFTY FIELDS

Per Cent Fat in Sections

CASE NUMBER	GROUP I	GROUP II	PER CENT DIFFERENCE*
1	0.0	0.0	0
2	3.4	3.4	0
3	14.4	14.9	3
4	16.5	16.8	2
5	21.3	23.1	8
6	27.4	27.7	1
7	34.0	36.4	7
8	37.6	38.4	2
9	46.7	43.4	7
10	50.8	53.6	6

* Percentage difference between estimates based on study of first (I) and second (II) consecutive groups of 50 fields, calculated by the formula:

$$\frac{\text{Difference (I - II)}}{\text{Estimate I}} \times 100$$

TABLE 2

COMPARISON OF ESTIMATES OF FAT CONTENT BASED ON A STUDY OF IDENTICAL FIELDS BY TWO DIFFERENT OBSERVERS

Per Cent Fat in Sections

CASE	OBSERVER L. B.	OBSERVER A. R. A.	PER CENT DIFFERENCE*
1	0.7	0.7	0
2	14.5	15.2	5
3	14.6	15.0	3
4	22.9	21.9	6
5	27.6	27.7	1
6	28.6	29.7	4
7	30.6	28.6	4
8	35.6	39.1	10
9	37.2	39.4	6
10	37.9	39.9	5

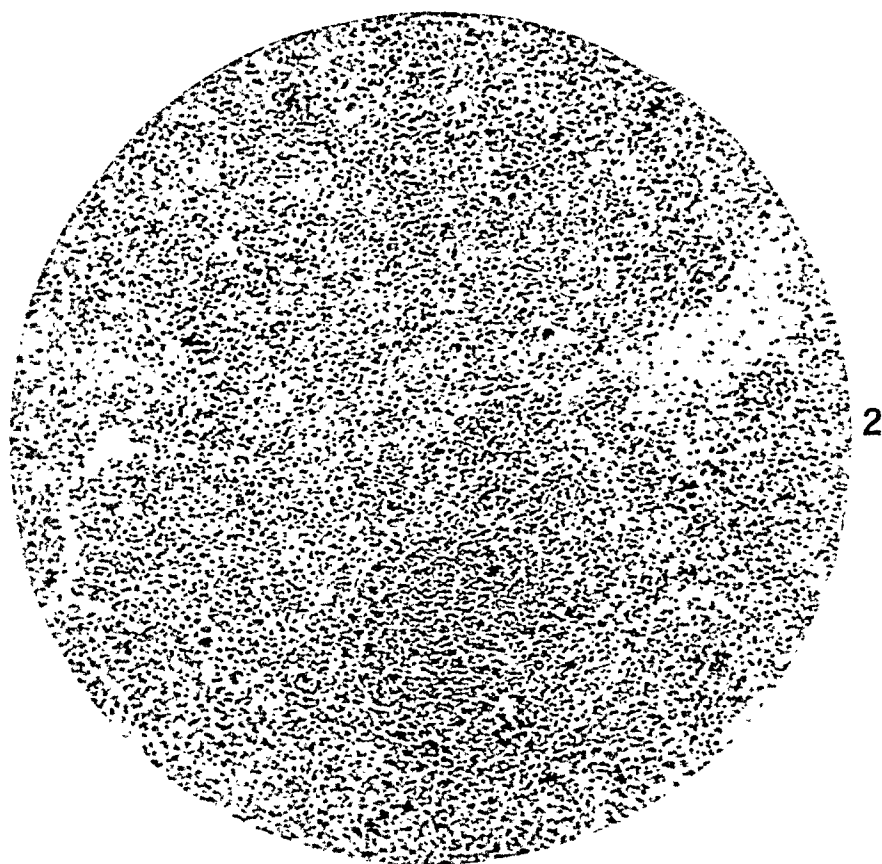
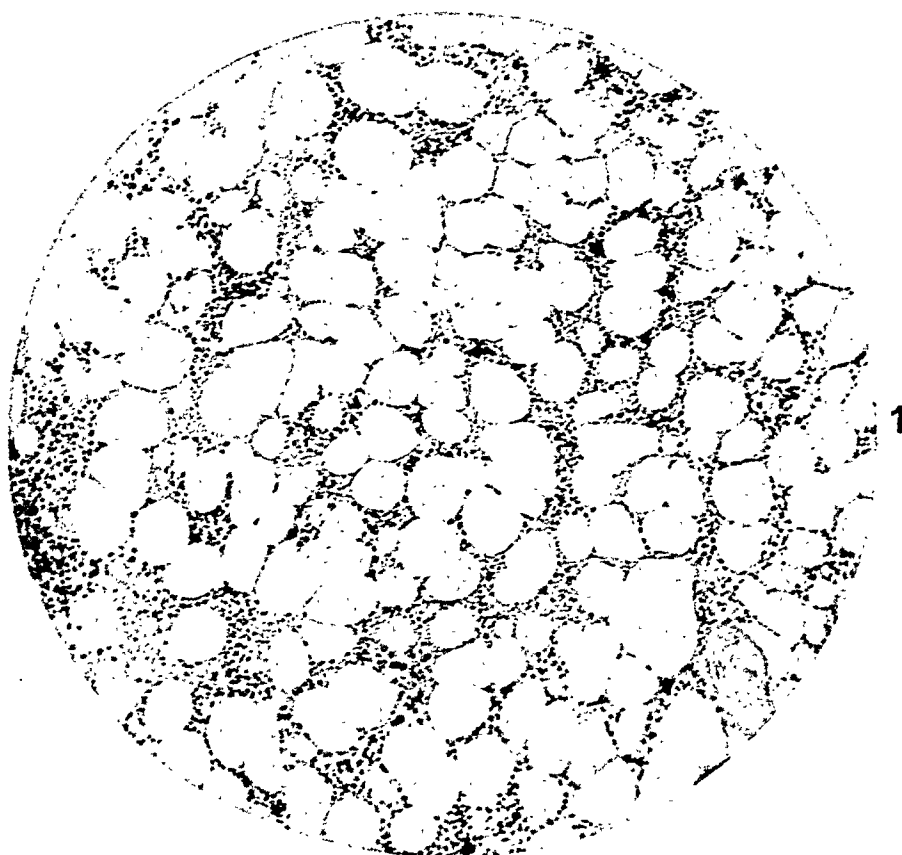
* Percentage difference between estimates by two different observers, L. B. and A. R. A., calculated by the formula:

$$\frac{\text{Difference (L. B. - A. R. A.)}}{\text{Estimate (L. B.)}} \times 100$$

of microscopic fields. Every tenth field was studied. It was found that the estimates based on 10 fields did not differ significantly from those based on 200 fields. Table 1 shows the results of a comparison of estimates of fat content of

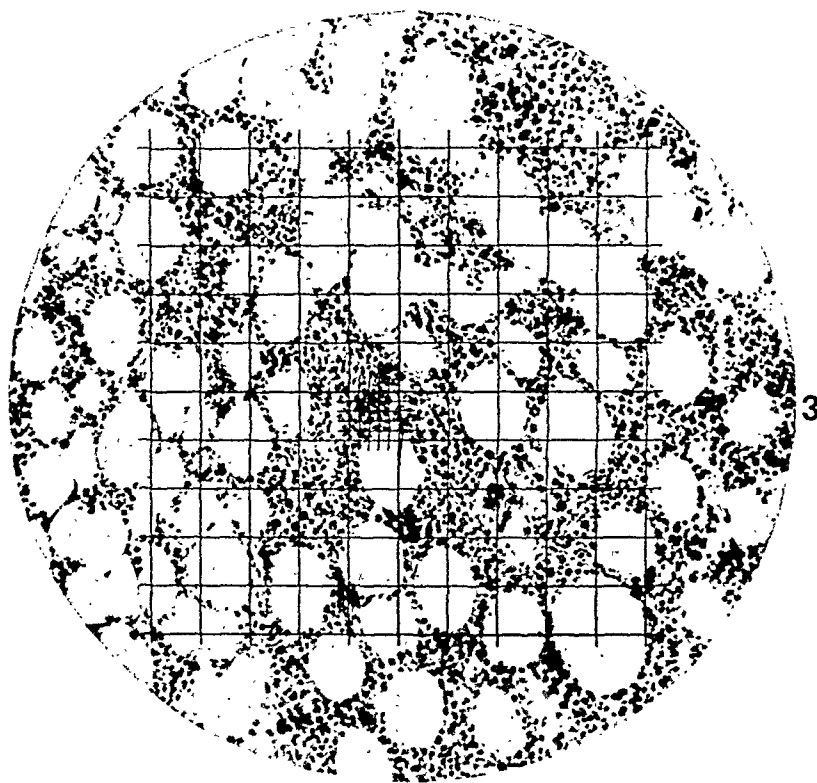
FIG. 1. Photomicrograph of section of marrow particles from a moderately fatty marrow.

FIG. 2. Photomicrograph of section of marrow particles from a cellular marrow (same magnification as Fig. 1).



the sectioned material based on a study of the first and second consecutive groups of 50 fields.

Since the differences observed were less than 10 per cent, the estimates used in this study were based on observation of 100 fields, although 50 fields would have been adequate. Because single fat spaces usually occupy more than one ruled square of the Whipple disc (Fig. 3), it is necessary to estimate fractions of fat covered squares. The possibility of personal error due to variance in estimating such fractions was considered. Hence, a survey was made to determine the magnitude of such variation among the results obtained by the two present authors (Table 2).



Photomicrograph showing appearance of field with superimposed Whipple eyepiece disc.

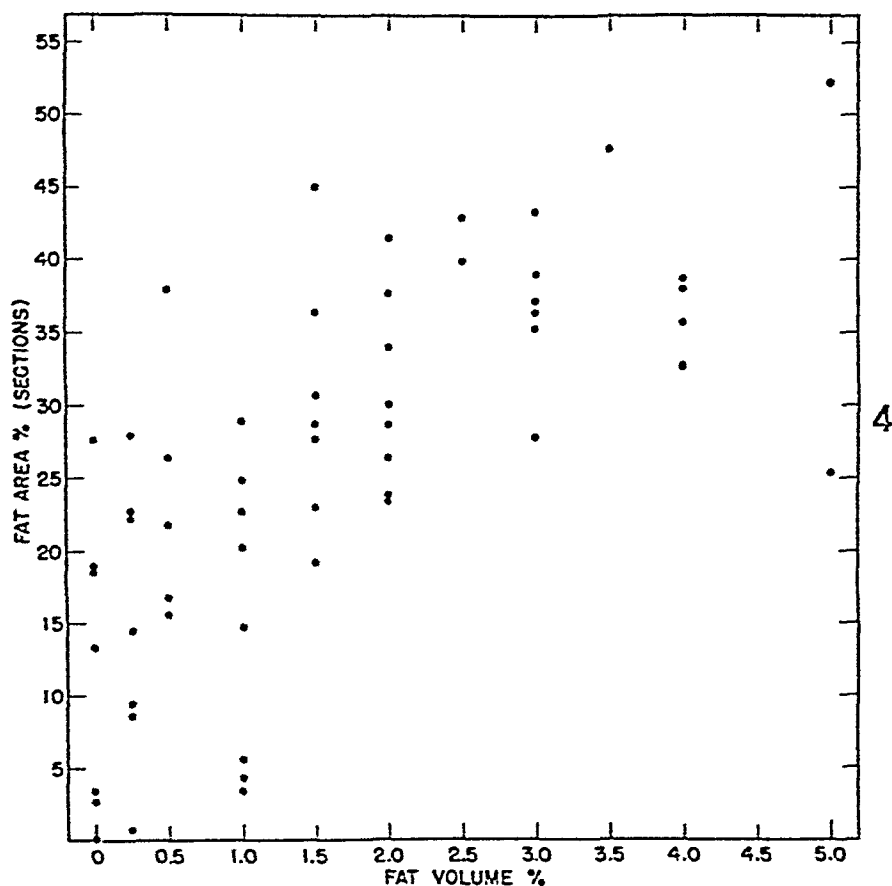
DISCUSSION

The results of the comparison between estimates of relative fat content of aspirated sternal marrow obtained by the volumetric method and those obtained by study of the sections of particles of marrow are indicated in the chart (Fig. 4).

Schleicher's⁶ study of 12 healthy males, between the ages of 18 and 40 years, yields a range of from 0.5 to 3.0 per cent of fat as determined by the volumetric method. It has been stated that pathologic fat metabolism may be detected by the volumetric method.⁶ Limarzi, Jones, Paul and Poncher¹ found a mean fat volume of 3.2 per cent in 10 normal persons, and a range of mean volumes of from 1.5 to 8.4 per cent in various pathologic conditions. Moosnick, Schleicher

and Peterson⁵ found a fat volume of 10 per cent in a patient with Addisonian pernicious anemia who was refractory to liver extract therapy. After treatment with intravenous choline chloride there was a rise in the erythrocyte and hemoglobin levels, preceded by reticulocytosis, and a reduction of the fat volume to a level of 1 per cent.

It can be stated that the volumetric method of determining relative fat content of aspirated sternal marrow is suitable for very crude estimates, but the section method is necessary for determining relative fat content with greater accuracy.



Scatter diagram illustrating relationship between estimates of fat content of aspirated sternal marrow based on volumetric data and observations of sections of marrow particles. Four points are not charted. They are: 7.0, 37.8; 7.5, 38.6; 8.0, 74.4; and 49.0; 98.0; for fat volume per cent, fat area per cent, respectively.

Thus, our material shows that if volumetric readings of 2 per cent or less fat are obtained, 88 per cent of the samples show a relative fat content of 35 per cent or less by area and, if volumetric readings of 2.5 per cent or more fat are obtained, 90 per cent of the samples show a relative fat content of over 35 per cent by area. However, if more accurate analysis of fat content is required, the volumetric readings are not reliable indices, as is shown by the large variance of results obtained for any single volumetric level (Fig. 4). For example, the data illustrated for volumetric readings of 1.0 per cent fat show a range from 3.4 to 28.8 per cent fat as determined by the section method; for volumetric readings of zero fat, the sections yield a range of from 0 to 27.6 per cent fat. Similar wide ranges were observed for other individual volumetric readings.

The use of such data, whether obtained by volumetric analysis or the method of examining sections of marrow particles, as outlined above, must be based on the determination of similar data in normal individuals of suitable age and sex, with rigid adherence to standardized methods of processing the material.

SUMMARY

An evaluation of volumetric data regarding the relative fat content of aspirated sternal marrow has been carried out by comparing the results obtained by centrifugation of the marrow sample with those obtained by examining sections of marrow particles.

It has been shown that the volumetric method is useful for crude estimates of relative fat content of samples of aspirated sternal marrow, but that it is not satisfactory for determining smaller variations of fat content. Marrow samples with fat volume percentages of 2 per cent or less yield fat area percentages of 35 per cent or less in approximately 90 per cent of the cases; marrow samples with fat volume percentages of 2.5 per cent or more yield fat area percentages of over 35 per cent in 90 per cent of the cases. However, the range of estimates of fat area percentages based on sectioned material, for any given volumetric determination, is very large.

For estimation of relative fat content of aspirated sternal marrow, the combined use of volumetric data and sectioned material is superior to the use of volumetric data alone.

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EVALUATION OF VOLUMETRIC DATA OBTAINED BY CENTRIFUGATION OF ASPIRATED STERNAL MARROW OF ADULTS.

II. ESTIMATION OF CELLULARITY OF STERNAL MARROW*

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*From the Departments of Pathology and Medicine, Wayne University College of Medicine,
and the Anemia Laboratory, Out-patient Department, Harper Hospital, Detroit*

In a previous communication² an evaluation of the estimation of relative fat content of aspirated sternal marrow of adults was presented. In this paper we wish to state the results of a study of the relative cellularity of samples of such material.

MATERIAL AND METHODS

The sternal marrow data and material from the group of 62 cases previously analyzed for fat content² were used in the present study. Our objective was to determine whether there is a significant correlation between the height of the nucleated cell layer in the centrifuged specimen (myeloid-erythroid volume, M-E volume⁷) and the degree of cellularity as estimated by inspection of the sections of particles of marrow from the same sample. It was observed that, in general, the areas occupied by cellular tissue in the sections are the approximate reciprocals of the areas occupied by fat. In some marrows, however, the non-fatty tissue in the sections is made up of vascular spaces, degenerated or "empty", gelatinous marrow spaces, fibrous tissue, or focal areas of necrosis. Accordingly, such marrows were not used for this study. This is justifiable because the question to be answered is, "Does the nucleated cell volume (M-E volume) of centrifuged aspirated sternal marrow provide an index of the cellularity of the sample of marrow?" If it does, the nucleated cell volumes and the cellularity of the sectioned material should be correlative. The relative cellularity of the sections (cellular area per cent) was calculated as the difference between the total area and the fat area in per cent as previously determined² in 36 cases.

DISCUSSION

The results of the comparison between the estimates of relative cellular content of aspirated sternal marrow obtained by the volumetric method and those obtained by study of the sections of particles of marrow are indicated in Figure 1.

The desirability of obtaining quantitative data from aspirated marrow has been stated by various authors.^{6, 11, 14} Many papers contain references to the M-E volume of centrifuged material in normal and diseased persons^{3, 6, 7, 8, 9, 10, 11, 12, 13, 15} but there has been little discussion of the reliability of such data. Reich and Kolb¹⁴ stated: "The cellularity of the marrow can be estimated roughly by doing a total nucleated cell count on the aspirated mixture or by examining the thickness of the buffy coat on the centrifuged specimen."

* Received for publication, May 7, 1947.

They point out that in doubtful cases sectioned material obtained by trephine of the sternum is necessary to supplement the information derived from sternal puncture. As we have shown, satisfactory material for preparing histologic sections may be obtained by aspiration.^{1, 15} The interpretation of the M-E volume has been commented on by Hebbel,⁴ who stated that it was of limited value in any one instance, since it will vary not only with hyperplasia and hypoplasia of the marrow but with the amount of sinusoidal blood which is obtained at aspiration. Isaacs⁵ is of the opinion that sections are indispensable for determining the architecture and arrangement of the bone marrow, as well as the determination of the relative distribution of fat and cellular tissue.

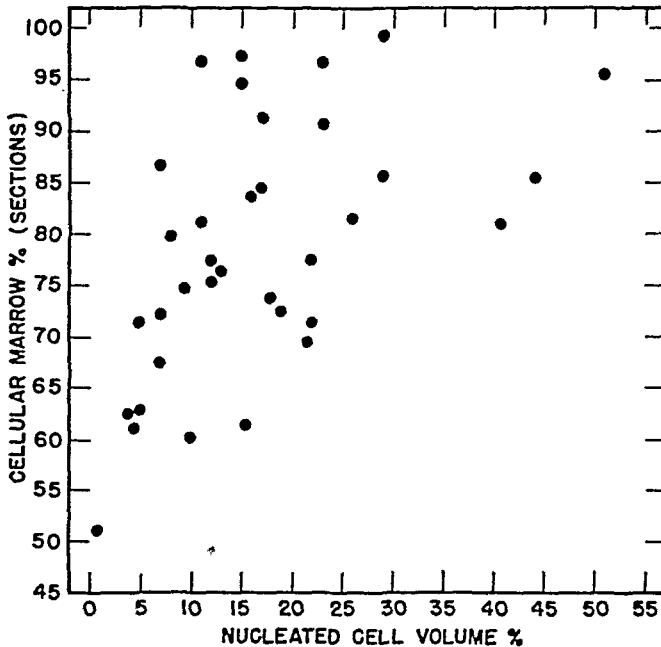


FIG. 1. Scatter diagram illustrating relationship between estimates of cellular content of aspirated sternal marrow based on volumetric data and observations of sections of marrow particles.

On the other hand, in a study of human sternal bone marrow in hyperthyroid and myxedematous states, R. M. Jones⁶ made extensive use of volumetric data in an attempt to reveal the changes in cellularity of the marrow in various patients with abnormally low or high basal metabolic rates, as compared with his observations on normal individuals. In 18 normal individuals, he found a mean M-E volume of 6.2 per cent. In 12 persons with hyperthyroidism, this value was 13.5 per cent, and in 7 persons with hypothyroidism the value was 2.4 per cent. The author concluded that in hyperthyroidism the marrow is hyperplastic, and that in hypothyroidism the marrow is hypoplastic. However, in his Chart 4 showing the effect of thyroid therapy on hypothyroid marrow (5 cases illustrated), three out of eight aspirations from patients with pathologically low basal metabolic rates yielded M-E volumes within the range he determined on normal individuals. In his Chart 5 showing the reaction of hyperthyroid marrow to

withdrawal of thyroid stimulation (4 cases illustrated), one of five aspirations from patients with pathologically high basal metabolic rates yielded an M-E volume within the range of his normals. Although the mean M-E volumes from hypothyroid and hyperthyroid patients were lower than normal, and higher than normal, respectively, it is doubtful whether the conclusions made by Jones are justified, because the range of values was ignored.

Figure 1 indicates that the mean cellular area percentage in the sections of marrow particles in cases yielding M-E volumes of 10 per cent or less, is 69.2 per cent, and that the mean cellular area percentage in the material yielding M-E volumes over 10 per cent is 83 per cent. However, the range of cellular area percentages based on sectioned material, for any given volumetric determination, is very large. For example, the data illustrated for volumetric readings of 0 to 5 per cent M-E layer show a range of from 51 to 87 per cent cellular area as determined by the section method; for volumetric readings of 21 to 25 per cent M-E layer, the sections yield a range of from 69 to 97 per cent cellular area. Similar wide ranges were observed for other volumetric readings. In other words, a low mean M-E volume is indicative of a relatively low mean cellularity of the sample, and a higher mean M-E volume is indicative of a relatively higher mean cellularity. Nevertheless, for any given case there is too great a range of possible M-E volumes to permit the use of these data as indices of relative cellularity of individual marrow samples. However, in clinical studies in which the values of mean M-E volumes of groups of cases are subjected to statistical analysis (which indicate the range of values observed), the information obtained by volumetric methods may be useful.

SUMMARY

An evaluation of volumetric data regarding the relative cellularity of samples of aspirated sternal marrow has been carried out by comparing the results obtained by centrifugation with those obtained by examination of sections of marrow particles.

It has been shown that the volumetric method may be useful for estimating the mean cellularity of a group of cases, but that the M-E volume, in any single case, is not an accurate index of the relative cellularity of the sample. Marrow samples with M-E volumes of 10 per cent or less yielded mean cellular area percentages of approximately 69 per cent; marrow samples with M-E volumes of over 10 per cent yielded mean cellular area percentages of 83 per cent. However, the range of estimates of cellular area percentages based on sectioned material, for any given volumetric determination, was very large.

The M-E volume cannot be used to determine the relative cellularity of individual samples of aspirated sternal marrow, although mean M-E volumes, properly analyzed, may be of value in interpreting differences among various groups of cases.

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MODIFIED EOSIN-METHYLENE BLUE AGAR AS A SELECTIVE MEDIUM FOR THE PRIMARY ISOLATION OF PATHOGENIC INTESTINAL BACTERIA*

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From the Mallory Institute of Pathology, Boston City Hospital, Boston

An unusually satisfactory solid medium for the examination of stools to determine the possible presence of pathogenic intestinal bacteria has been used in this laboratory for several years. In the past, eosin-methylene blue agar has been used only in conjunction with other mediums for the isolation of fastidious strains of salmonellae and shigellae, which tend to be inhibited by the more strongly selective mediums such as SS (Shigella-Salmonella) agar, desoxycholate citrate agar and bismuth sulfite agar. Various methods of increasing the selectivity of eosin-methylene blue agar which would not make it more inhibitory have been recommended. These usually consist of the addition of other dyes, such as brilliant green (Knox *et al.*)² The latest modification to appear in the literature is that of Felsenfeld and Young¹ who recommend the addition of small amounts of sodium citrate and picric acid to the basic medium. The method to be discussed in this paper is somewhat less complex in that it consists merely in the change of pH of the basic medium.

MATERIALS AND METHODS

The basic medium is Levine's Eosin-Methylene Blue Agar (Difco) which has the following formula:

Bacto-Peptone.....	10.0	gm.
Bacto-Lactose.....	10.0	gm.
Dipotassium phosphate.....	2.0	gm.
Bacto-Agar.....	15.0	gm.
Bacto-Eosin Y.....	0.4	gm.
Bacto-Methylene Blue.....	0.065	gm.
Distilled water.....	1000.0	cc.

According to the manufacturer, the final pH of this medium is approximately 7.1. The pH is altered before autoclaving by the addition of normal sodium hydroxide solution (usually 1.5 cc. per liter of medium) so that the final pH of the agar is approximately 8.0. The pH of each new lot of desiccated medium is determined electrometrically, but as a rule little deviation from the above quantity of sodium hydroxide solution is found necessary. The medium is poured into standard Petri dishes in amounts not greater than 12.5 cc. per plate.

To inoculate the agar, a heavy suspension of the stool is made, usually in the container in which it is received, by the addition of a few cubic centimeters of saline or broth to an appropriate portion of the specimen. Three to six loopfuls of this suspension are streaked on one plate; then two successive plates are also

* Received for publication, April 17, 1947.

inoculated without flaming the loop. The plates are incubated from eighteen to twenty-four hours at 37 C. and then examined. Colorless colonies are picked (usually from the second or third plate) to determine whether or not they are composed of pathogenic organisms. At the time of the initial culture, approximately 1 gm. of feces is emulsified in tetrathionate broth enrichment medium. After incubation from eighteen to twenty-four hours two or three loopfuls of the enrichment, including some of the sediment from the bottom of the tube, are inoculated on a single eosin-methylene blue agar plate. If pathogens are recovered from the initial culture, nothing further is done with this plate. Otherwise colorless colonies are picked and identified as in the primary isolation.

TABLE 1

COMPARISON OF MODIFIED EOSIN-METHYLENE BLUE (EMB) AGAR WITH DESOXYCHOLATE CITRATE (DC) AGAR

Number of stools cultured.....	92
Total number enteric pathogens recovered.....	12
<i>E. typhosa</i>	3
Strains of <i>Salmonella</i>	8
Strains of <i>Shigella</i>	1
Number of enteric pathogens recovered with EMB.....	12
Number of enteric pathogens recovered with DC.....	9*

* All pathogens recovered selectively on EMB were strains of *Salmonella*.

TABLE 2

COMPARISON OF MODIFIED EOSIN-METHYLENE BLUE (EMB) AGAR WITH SS AGAR

Number of stools cultured.....	76
Total number enteric pathogens recovered.....	10
<i>E. typhosa</i>	1
Strains of <i>Salmonella</i>	9
Strains of <i>Shigella</i>	0
Number of enteric pathogens recovered with EMB.....	9*
Number of enteric pathogens recovered with SS Agar.....	9*

* 1 stool positive on EMB was negative on SS, and 1 stool positive on SS was negative on EMB.

In the past, eosin-methylene blue agar modified as described above was compared with Endo's agar and found to be equally satisfactory. Since the former is easier to prepare and since it can be stored without unusual precautions, it has been used instead of Endo's medium.

Modified eosin-methylene blue agar recently has been compared in this laboratory with two other solid mediums commonly used for primary isolation of intestinal pathogens from feces, desoxycholate citrate agar and SS agar.

Ninety-two simultaneous cultures were made on modified eosin-methylene blue and desoxycholate citrate agars (Table 1). Not only did the desoxycholate agar give consistently poorer results, but also was found to be as difficult to prepare and to preserve for later use as Endo's medium. With two stools it gave a

better growth of the pathogen than the eosin-methylene blue agar, but frequently plates showed confluent growth and many times there was imperfect color differentiation of the colonies.

Seventy-six simultaneous cultures were made on SS agar and modified eosin-methylene blue agar. SS agar gave as uniformly good results as did the modified eosin-methylene blue agar. The former medium is easily prepared and keeps well. Both of the pathogens isolated on but one of these two mediums as noted under Table 2 were group B strains of *Salmonella*.

DISCUSSION

Formerly, when standard eosin-methylene blue agar plates were read, the numbers of pink colonies of indeterminate types were numerous and hence the medium fell into disfavor except for its use in attempts to isolate fastidious strains of pathogens. It was considered that the pH of the medium might be the factor which caused poor color differentiation; hence cultures at various levels of pH were made. At a pH of 8.0, it was found that there was little inhibition of growth, but that the selective coloring of nonpathogenic colonies was more satisfactory than at the usual pH of 7.1. At a pH of 8.0, colonies of *Escherichia coli* are uniformly blue-purple with a green sheen. *Acrobacter aerogenes* grows in large, glistening, pink colonies with purple centers. Colonies of the paracolon group are usually pale pink to colorless as are those of *Proteus morganii*. The spreading species of *Proteus* are largely inhibited, but slight halos are always apparent about their colonies. *Pseudomonas aeruginosa* grows in deep lavender-pink colonies which are usually easily identified. *Klebsiella pneumoniae* grows in large, mucoid, pink colonies, usually with blue centers. Gram-positive organisms, if they grow, form tiny colonies much smaller than those of the gram-negative group. The colonies of the pathogens vary in size. Colonies of strains of shigellae are usually smaller than those of salmonellae, but all are pale blue to colorless, as are those of *Alcaligenes faecalis*. Hence with the initial culture on this medium, one rarely picks organisms other than pathogens, organisms of the paracolon group, *Alcaligenes faecalis* and *P. morganii*.

As noted above, it is felt that the results obtained with SS agar are as satisfactory as those with modified eosin-methylene blue agar. The use of the latter, however, has certain advantages in a laboratory such as this where the majority of cultures are not of stools. To be able to use a medium for the culture of stools which can also be used for the routine identification of gram-negative organisms from other sources, such as the urine, is a distinct advantage. It is believed that the fewer mediums that have to be prepared for routine bacteriologic work, the more uniform they are likely to be. Likewise, the fewer mediums that are used, the more accurate the macroscopic identification of colonies when plates are examined, after one has become thoroughly familiar with the mediums in use.

On the other hand, in a laboratory where bacteriology is largely confined to the study of enteric pathogens, it is believed that the use of SS agar and an enrichment medium is probably more satisfactory.³ SS agar can be inoculated directly from the stool or from a rectal swab at the bedside. It requires the use

of but one plate. It is also somewhat less expensive (approximately 13 per cent cheaper to use).

SUMMARY

A new modification of eosin-methylene blue agar is presented and its use as a medium for the primary isolation of enteric pathogens is discussed. Its efficiency is compared with desoxycholate citrate agar and SS agar. It is recommended that its use be supplemented by an enrichment medium such as tetrathionate broth in laboratories where routine bacteriology is performed.

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DILUTION OF CAPILLARY BLOOD IN THE MICROANALYSIS OF PROTEIN*

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In a study of blood proteins in children with nephrosis, it became necessary to devise a technic by which determinations of total protein, albumin and globulin could be made on single samples of blood taken by capillary puncture. A satisfactory micromethod, which utilizes a Bauer and Schenck tube for the dilution of blood, was developed for repeated analyses on the same patient. One-tenth ml. of whole blood is added, immediately after its withdrawal, to 2.9 ml. of 0.9 per cent sodium chloride in the Bauer and Schenck tube. For subsequent handling of this diluted blood, a variety of existing methods was tested. Colorimetric methods were applicable only to the screening of normal patients from those with disease; a semimicro-Kjeldahl analysis was found to be the only accurate method for studying opalescent serums from treated patients.

PROCEDURE

The patient should be in an anteprenal state with a limited fluid intake;³ however, a postabsorptive state of about four hours is satisfactory. A 0.02 per cent dilution of blood or serum in saline may be prepared from blood withdrawn by venipuncture, but it is preferable to take each sample of blood directly from the patient.

Into a Bauer and Schenck centrifuge tube, 2.9 ml. of 0.9 per cent saline is delivered slowly from a Koch microburet; about four minutes is allowed for drainage. This liquid should not be forced into the capillary base of the tube. Keeping the tubes stoppered throughout the procedure prevents both evaporation and denaturation.

Preparing for plasma dilution (P.D.). From a capillary puncture of the finger or ear, 0.1 ml. of whole blood is drawn into a Folin pipet† and is delivered into the saline in the Bauer and Schenck tube while keeping the tip of the pipet above the surface of the liquid to prevent any loss by overflow. After the pipet is rinsed with the diluting fluid until clear of blood, the tube is inverted over the thumb and shaken to separate the plasma from the cells. It is then placed in a wooden holder and centrifuged for twenty minutes at 2000 R.P.M. The cell volume (C.V.) is recorded by noting the height of the column of packed cells which should be estimated between divisions to 0.001 ml. Calculation:

$$\text{P.D.} = \frac{0.1 - \text{C.V.}}{3.0 - \text{C.V.}}$$

* Received for publication, April 14, 1947.

† The pipet graduated in 1/100 ml., although awkward to handle, gives exact duplications in readings of cell volume (C.V.). A variation of 0.001 C.V. gives a difference of 0.2 gm. per cent of total protein.

Of the 2.5 ml. of diluted plasma available for study, 1 ml. is used for total protein (T.P.) analysis, while a second ml. is available for albumin-globulin separation.

Albumin-globulin separation. To 1 ml. of the plasma dilution in a test tube is added 4 ml. of filtered 27.5 per cent aqueous solution of C.P. Baker's special anhydrous powdered sodium sulfate for nitrogen determination.* Then, an equal volume of washed ether is added to decrease the density of globulin. The tube is stoppered tightly and shaken vigorously for twenty seconds.⁴ Following centrifugation at 2000 R.P.M. for ten minutes, a thin globulin pellicle can be noted at the interface; a thick pellicle indicates that too little ether has been used. A clean 4 ml. volumetric pipet must be employed for each tube to remove the supernatant fluid for albumin determination to a Kjeldahl flask. The globulin is analyzed, after the ether is poured off, by quantitatively washing the pellicle into a Kjeldahl flask. The ether fraction is nitrogen-free.

Choice of subsequent procedure. Technical details have been worked out for only three of the standard methods, the Kjeldahl, a tyrosine colorimetric procedure, and an adaptation of the Heidelberger and MacPherson¹ method which measures both tyrosine and biuret configurations. Although the Kjeldahl method is time-consuming and requires special apparatus, the details of this technic are outlined to define the criteria for digestion and distillation.

Kjeldahl Analysis

For digestion, the sample is pipetted into a 100 ml. Kjeldahl flask fitted with a female ground glass joint. Added in succession are: a glass bead to prevent bumping; 1 ml. of digestion mixture⁷ (30 gm. potassium sulfate, 5 gm. copper sulfate, 500 ml. distilled water, 480 ml. concentrated sulfuric acid and 0.5 gm. selenium metal); and 5 ml. of distilled water to wash down the sides. Digestion on a 6 unit microdigestion shelf is continued one-half hour after clearing.

An all-glass apparatus[†] modified from that of the Pregl steam technic⁶ is employed for distillation of the digest in the Kjeldahl flasks. The apparatus is steamed through with distilled water until 80 ml. is collected. Blanks distilled at the beginning and end of each series should be nitrogen-free (pale violet) and thus require no titration. Each Kjeldahl flask is again washed down with 5 ml. of distilled water and is attached at the male joint. Ten ml. of 40 per cent aqueous sodium hydroxide prepared from technical flakes is added slowly; distillation into 10 ml. of receiving fluid (5 per cent aqueous boric acid containing 5 ml. indicator solution per liter of boric acid;⁸ the indicator is 100 ml. 0.03 per cent methyl red in 95 per cent ethyl alcohol and 15 ml. 0.1 per cent alcoholic methylene blue) proceeds until 30 ml. is collected at the rate of 10 ml. per minute. The distillate is titrated with N/70 hydrochloric acid⁵ from a Koch microburet calibrated in 1/100 ml. One ml. of N/70 hydrochloric acid is equivalent to

* The solution concentration is 21.6 per cent.²

† The glass apparatus may be obtained from Mr. R. C. Kurowski, 104 Jones Street, Etna, Pittsburgh 23, Pennsylvania.

0.2 mg. nitrogen. Normally the plasma dilution contains ± 0.2 mg. nitrogen. Calculations:

Gm. per cent T.P. = (ml. HCl used to neutralize the sample—

$$\text{ml. HCl for blank}) \times 0.2 \times \frac{100}{\text{P.D.}} \times \frac{6.25}{1000}$$

Gm. per cent globulin = same as for total protein

$$\text{Gm. per cent albumin} = (\text{ml. HCl} - \text{ml. HCl for blank}) \times \frac{1}{0.8}$$

$$\times 0.2 \times \frac{100}{\text{P.D.}} \times \frac{6.25}{1000}$$

DISCUSSION

Repeated analyses have been made on more than 100 bloods from patients at the Children's Hospital of Pittsburgh and on 200 samples from normal persons. Antibody nitrogens and nitrogen of macromolecular substance for experimental animal injection were determined also. I believe that the accuracy of the method is dependent, in a large degree, upon the apparatus employed. With nitrogen-free blanks, the accuracy of the Kjeldahl method is determined by the bore of the buret tip; if a tip delivers 0.015 ml. per drop, that quantity, in effect, represents the greatest accuracy that can be expected. Macro- and micro-Kjeldahl analyses on the same sample of blood were in agreement to within ± 0.01 to 0.2 gm. per cent total protein.

In any ultramicrotechnic the mathematical factor is magnified by any discrepancy in the precision with which the measurements of blood are made. This applies to the method under discussion as well as to those methods in which serum is separated in a capillary tube.

Albumin determinations were not always so exact as those of total protein. This difference is attributed to the difficulty in pipeting the subnatant fluid without the introduction of some ether. A source of error which presented a problem was the increased atmospheric nitrogen due to the presence of animals near the room where proteins were being analyzed. Even uncovered urine in bottles in the laboratory was responsible for an increase in the nitrogen in the blanks.

SUMMARY

A technic is presented which introduces a saline dilution of 0.1 ml. of whole blood from which total protein, albumin and globulin values may be obtained by using adaptations of existing methods.

Acknowledgment: The assistance of Miss Esther Graff, M.T.(A.S.C.P.), and Miss Ruth Flanagan, B.S., is gratefully acknowledged.

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RAPID METHOD FOR PRODUCING SUSPENSIONS OF FLAGELLAR ANTIGENS AND FOR INDUCING PHASE SUPPRESSION*

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A simple procedure for obtaining suspensions of "H" antigens of either phase has been used successfully by the writer. A tube half-full of semisolid medium and with a narrow opening in the bottom, is placed in a tube of broth. Motile organisms migrate down from the surface of the semisolid medium, through the opening in the bottom of the inner tube and into the broth. Thus, selection of motile forms and their multiplication in the broth occur in the same over-night incubation period with no separate handling required. Phase suppression occurs when serum of the phase to be suppressed is mixed with the semisolid medium. The method differs from others in that the suspensions are available within twenty-four hours after preparation is begun,¹ awkward manipulations are eliminated, and the assembly can be stored conveniently for immediate use.^{1,2} Only ordinary equipment is required.

MATERIALS

The following materials are prepared:

1. Pieces of glass tubing, 100 x 8 mm., each with one end softened in a flame until it has an opening of 1 mm. or less. Tubing is sterilized with the narrow opening down, in test tubes.
2. Screw-capped test tubes, 125 x 16 mm., containing 6 ml. of sterile semisolid medium.¹
3. Screw-capped test tubes, 125 x 20 mm., containing 8 ml. of sterile refrigerated tryptose-phosphate broth (Difco).

METHOD

Using heated forceps, insert a piece of the sterile glass tubing into a tube of the melted semisolid medium. With the amounts of mediums and sizes of tubes given above, the inner tube becomes half-full of medium. The assembly is cooled and then refrigerated just long enough (ten minutes) to congeal the medium so that it will remain inside the inner tube during transfer to a tube of broth. Longer refrigeration causes fragments to stick to the outside of the tube upon withdrawal. To transfer the inner tube, it is held stationary with heated forceps, while the outer tube is revolved several times with the other hand. The inner tube is then withdrawn and placed in a tube of refrigerated broth. With the amounts of mediums and sizes of tubes given above, the levels of semisolid medium and broth should closely coincide. If they do not, broth should be added or removed with a 1 ml. pipet until its level is identical with that of the

* Received for publication, April 4, 1947.

semisolid medium in the inner tube. This should be done while the mediums are still cold. The assembled tubes may be stored and are ready for immediate use. Screw caps minimize evaporation.

To test a culture for motility and to obtain a suspension of flagellar antigen, the surface of the semisolid medium in the inner tube is merely pricked with an inoculating needle and the assembly is incubated. Nonmotile organisms remain at the surface of the semisolid, whereas motile forms migrate down through it and into the broth which becomes cloudy. On the following day, the tubes are refrigerated from ten to fifteen minutes to congeal the semisolid medium, and the inner tube containing it is then removed with heated forceps and discarded. Leakage from the inner tube into the broth during removal does not occur if the mediums are cold. An equal quantity of formalinized saline is added to the residual broth before testing for "H" antigens.¹ Enough suspension is available for ordinary purposes and more will be available on the next day if the broth is subinoculated before adding saline.

When phase-suppression is desired, two loopfuls of the serum corresponding to the phase to be suppressed are mixed with a tube of the melted semisolid medium which has been cooled to 50 C. A piece of the glass tubing is inserted aseptically and the same procedure is followed as before. Organisms of the suppressed phase are retained near the top of the semisolid medium, while those of the alternate phase migrate down and out into the broth.

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USE OF P-NITROPHENYLPHOSPHATE AS THE SUBSTRATE IN DETERMINATION OF SERUM ACID PHOSPHATASE*

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The estimation of acid phosphatase in serum depends upon the determination of the rate of hydrolysis of a monophosphoric acid ester in a suitable buffer. Disodium phenyl phosphate and sodium glycerophosphate have been the two substances commonly used with the subsequent determination of the liberated phenol and phosphate respectively.^{2,4} A procedure for alkaline phosphatase has recently been published by Bessey, Lowry and Brock¹ in which p-nitrophenylphosphate is used as the substrate and the liberated p-nitrophenol is determined directly in a photo-electric colorimeter. The method is recommended for both the micro- and macro-determination of acid and alkaline phosphatase, but neither the procedure nor normal values is included for the acid phosphatase. The determination of alkaline phosphatase by this method has proved so satisfactory that the estimation of the acid phosphatase was undertaken using this substrate.

PREPARATION OF REAGENTS

Reagent A. Sorensen's M/10 citrate-hydrochloric acid buffer, pH 4.8. Dissolve 21.008 gm. of citric acid in water in a liter flask and add 200 ml. of 1 N sodium hydroxide. Dilute to volume. To 900 ml. of this solution add 100 ml. of N/10 hydrochloric acid.

Reagent B. Prepare a 0.4 per cent solution of disodium p-nitrophenylphosphate in 0.001 N hydrochloric acid. According to Bessey,¹ the reagent contains 50 per cent inert material so that double the quantity should be used or the reagent should be recrystallized before use. If the solution has a definite yellow color, it should be extracted with ether to remove free p-nitrophenol.

Substrate. Mix equal parts of reagents A and B. Store in the refrigerator or in the frozen state. Some hydrolysis may take place with standing and the volume of reagent blank may increase. The substrate may then be extracted with ether to remove the free p-nitrophenol.

STANDARDIZATION OF PHOTO-ELECTRIC COLORIMETER

Prepare solutions containing 0.2, 0.4, 0.6, and 1.0 mM, respectively, of p-nitrophenol per liter. Since the millimol-unit is defined as "the phosphatase activity which will liberate 1 mM of p-nitrophenol per liter per hour",¹ these standards are equivalent to 0.4, 0.8, 1.2 and 2 units respectively, as the incubation period is only thirty minutes.

To 0.2 ml. of each standard measured into colorimeter tubes add 4.0 ml. of

* Received for publication, April 17, 1947.

0.1 N sodium hydroxide. The tubes are then read in a photo-electric colorimeter using a filter with a wave length between 400 and 420 $m\mu$. The values obtained are plotted against the units in the standard.

The millimol unit may be multiplied by 1.8 to convert to milligrams of phosphate liberated per 100 ml. of serum per hour.^{3, 4}

PROCEDURE

One ml. of substrate is measured into a colorimeter tube which is placed into a water bath at 38 C. When the temperature in the tube has reached the desired level, 0.2 ml. of serum is added and the mixture is incubated for exactly thirty minutes. The color is then developed by the addition of 3.0 ml. of 0.1 N sodium hydroxide, and the tube is read in the photo-electric colorimeter, using a filter with a wave length between 400 to 420 $m\mu$. A substrate and a serum blank are prepared. The former consists of 1 ml. of substrate and 3.2 ml. of

COMPARISON OF BODANSKY AND MILLIMOL UNITS OF SERUM ACID PHOSPHATASE

SERUM NUMBER	BODANSKY UNITS	MILLIMOL UNITS	RATIO
1	0.18	0.32	1.78
2	0.20	0.37	1.85
3	0.20	0.33	1.65
4	0.12	0.20	1.67
5	0.90	1.40	1.56
6	0.61	1.20	1.93
7	0.78	1.40	1.79
8	149.	70.	2.13
9	8.5	4.3	1.97
10	4.0	1.95	2.05
11	1.72	1.07	1.61
Average.....			1.81

0.1 N sodium hydroxide and the latter of 0.2 ml. of serum and 4.0 ml. of 0.1 N sodium hydroxide. Both blanks are subtracted from the reading of the unknown before calculation from the standard curve.

The quantity of serum used in the determination was chosen so that accurate readings could be obtained for the relatively low values usually found. If high values are encountered, and the quantity of p-nitrophenol liberated exceeds the amount which can be read on the standard curve, the unknown may be diluted to as much as 100 ml. and the reading made. The blanks are likewise diluted to the same volume and subtracted as usual. The substrate is present in sufficient quantity to give accurate values up to about 15 millimol units. When this value is exceeded, the determination should be repeated using a smaller quantity of serum and/or a shorter incubation period.

DISCUSSION

The activity of the acid phosphatase in a p-nitrophenyl-phosphate buffer was measured over a range of pH from 3.0 to 5.8 (glass electrode). The optimum

pH was at pH 4.9 to 5.0, the same as the optimum for the enzyme in other substrates.^{2,4} A variation of 0.1 pH. caused little variation in activity and the amount of hydrolysis was proportional to the time of incubation, up to three hours, when normal serums were used.

The blank correction for the color and density of the serum was relatively large with the amount of serum necessary to give reliable results in the normal range and the variation in the reading of the blank in acid and alkaline solutions was often appreciable even though the serum showed no apparent hemolysis. The greatest discrepancies occurred when the serum contained increased quantities of bilirubin. A separate serum blank was therefore used instead of acidifying the unknown after the initial reading and rereading as is done in the determination of alkaline phosphatase.¹

Normal values

Tests were run on a series of 25 women and 75 men in order to establish the normal limits of acid phosphatase activity in the serum in terms of the millimol unit. These patients were free from any condition, such as carcinoma of the prostate, which might cause an elevation of the acid phosphatase. The acid phosphatase activity of the serums of the women ranged from 0.01 to 0.56 units with an average of 0.23 units. The range for the men was from 0.03 to 0.63 units with an average of 0.27 units.

Comparison of units

The millimol units were compared with Bodansky units, expressed as mg. of phosphate liberated per 100 ml. of serum per hour, determined by a modification of the Shinowara technic.^{3,4} If the standard incubation period of one hour was used for the latter method, using normal serums, poor correlation was found between the two methods. If the incubation period was increased to three hours and the final result divided by three, better correlation was obtained (Table 1). The average ratio obtained between Bodansky units expressed as mg. phosphate liberated per 100 ml. of serum per hour and the millimol units was 1.81. Bessey¹ found the same value when he compared the Bodansky units for alkaline phosphatase and millimol units. Our highest normal value of 0.63 multiplied by this factor gave a value of 1.1 which is the upper limit of normal for the technic used for comparison.

SUMMARY

A procedure is outlined for the estimation of acid phosphatase in serum using p-nitrophenylphosphate as the substrate.

The normal values for the acid phosphatase activity of the serum of 25 women ranged from 0.01 to 0.56 millimol units with an average of 0.23 units. The range for 75 men was from 0.13 to 0.63 units with an average of 0.27 units. The product obtained by multiplying the number of millimol units by 1.8 gives a value equivalent to Bodansky units (mg. phosphate liberated per 100 ml. of serum per hour).

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STATISTICAL ERRORS IN COUNTING BLOOD CELLS*

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Apart altogether from mistakes in technic in the preparation of a specimen, any count on a hemocytometer is subject to errors arising from the chance distribution of the cells from square to square. While everyone knows that differences occur, few appreciate their significance in the final interpretation of the result. Possibly some are intimidated by the mathematics involved.

"Student"² showed that the standard deviation of a hemocytometer count could be approximated by the square root of the number of cells counted. Where two counts are being compared, the standard deviation of the difference between them is given by the formula:

$$S.D._{diff.} = \sqrt{S.D._1^2 + S.D._2^2}$$

where $S.D._1$ and $S.D._2$ are the standard deviations of the two counts. The probability of occurrence of statistical deviations relative to the standard deviation has been computed by Pearl,¹ a portion of whose table is given here for purposes of illustration.

SIGNIFICANCE OF DIFFERENCES

DIFFERENCE S. D. diff.	PROBABLE OCCURRENCE OF A DEVIATION AS GREAT AS, OR GREATER THAN, THAT DESIGNATED IN A HUNDRED TRIALS	ODDS (TO ONE) AGAINST THE OCCUR- RENCE OF A DEVIATION AS GREAT AS, OR GREATER THAN, THAT DESIGNATED
0.67449	50.00	1.00
1.0	31.73	2.15
2.0	4.55	20.98
3.0	.270	369.4
4.0	.00634	15,770
5.0	.0000573	1,744,000

The original tables should be consulted for the complete range.

An example may illustrate the principle. Does a count of 3200 cells per cu. mm. differ significantly from one of 5000 cells? If a single square millimeter, of depth 0.1 mm., of a 1:20 dilution has been examined, and if in the first case 16 cells were counted and 25 cells in the second, the computation would be:

$$S.D._1 = \sqrt{16} = 4 \quad \text{and} \quad S.D._2 = \sqrt{25} = 5.$$

$$\frac{\text{Difference}}{S.D._{diff.}} = \frac{25 - 16}{\sqrt{25 + 16}} = \frac{9}{\sqrt{41}} = \frac{9}{6.4} = 1.4.$$

From Pearl's tables we see that there is a 16.15 per cent probability that such a difference would occur by chance, or the odds are 5.19 to one against it. Few

* Received for publication, April 15, 1947.

people would care to make an assertive statement on such slender odds. If, however, the counts had been based on four such squares (with a multiplication

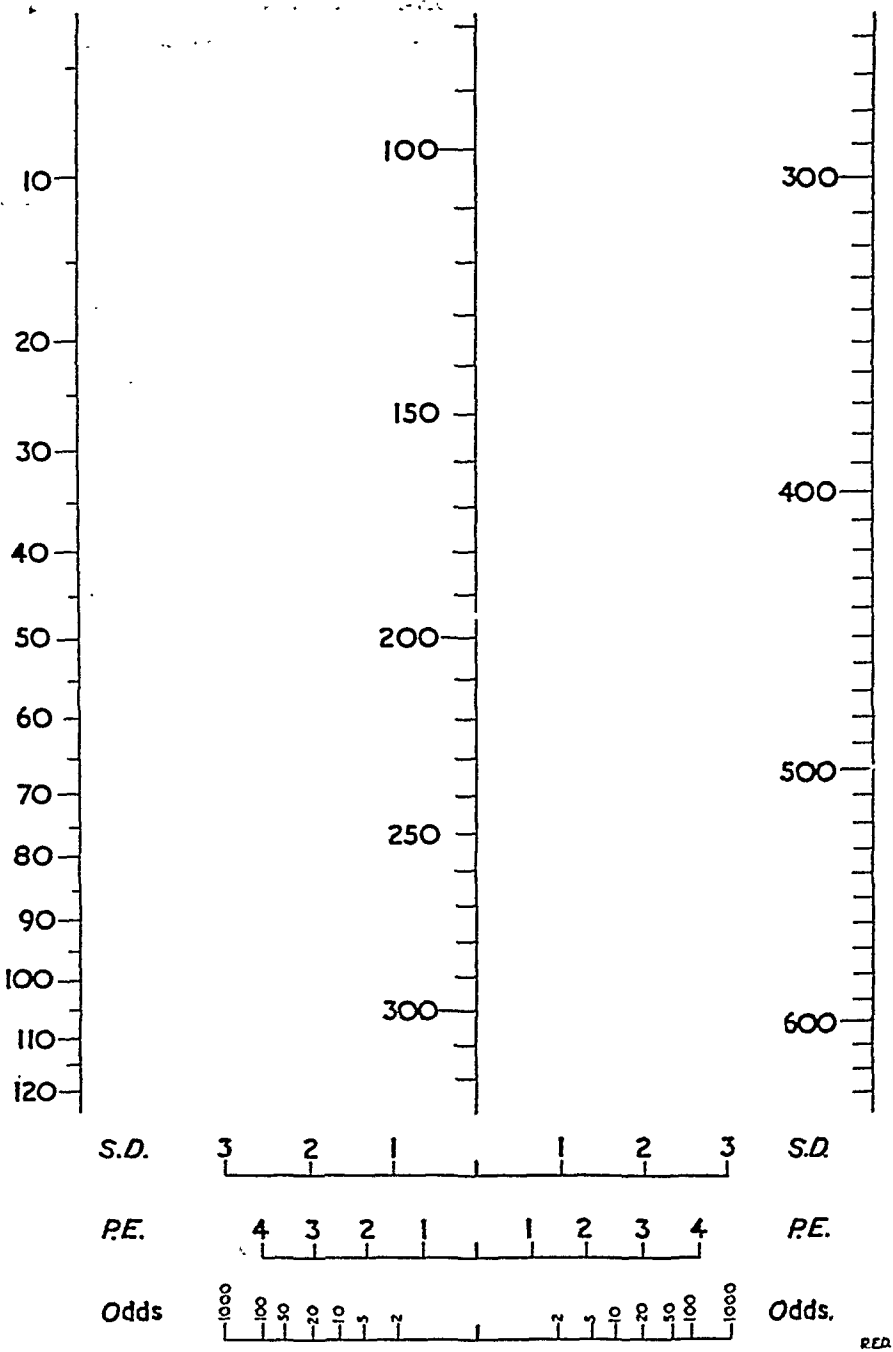


FIG. 1. Chart for estimating statistical significance of difference between two hemocytometer counts.

factor of 50 as against 200) the ratio between the difference and its standard deviation would be 2.5, and the odds against both counts being random varia-

tions of the same count would be 79.52 to one, which is a better basis for argument.

The mathematics of this is tedious, and it is for this reason that the accompanying chart (Fig. 1) was devised.

To use the chart, mark on the edge of a piece of card from the lines at the bottom of the chart, the desired calibration, selecting standard deviation (S.D.) probable error (P.E.) or odds, as desired. Place the center point of the calibration against one or other number of cells counted, and read off against the other number, the ratio Difference/S.D., the ratio Difference/P.E., or Odds, as the case may be.

Bear in mind that the volumes counted must be the same in each case, and that the figures used for comparison must be the actual number of cells counted, and not any calculation therefrom.

SUMMARY

A chart for the rapid estimation of the significance of the difference between two hemocytometer counts is shown.

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AGGLUTINATION OF ERYTHROCYTES IN HAYEM'S SOLUTION IN DISEASE*

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Agglutination of erythrocytes in Hayem's solution is noted frequently, yet little clinical significance was attributed to it until De Angelis and Huntsinger² published their article suggesting the use of this reaction as a test. Gradwohl³ mentioned this type of reaction occurring in three cases of Hodgkin's disease, but attached no clinical significance to it, except to emphasize the difficulty encountered in doing a red blood cell count. Other writers⁴ have observed this same phenomenon and Wintrobe⁵ noted clumping in cases of multiple myeloma, kala-azar, cirrhosis of the liver, pneumonia and nephritis.

The clumping of red blood cells in Hayem's solution has not been explained, but a study by Ch'u and Forkner¹ indicates that it is due to the formation of a coarse precipitate in the blood plasma. In our studies, the reaction occurred in a variety of infections, neoplastic and other diseases in which the patients were acutely or chronically ill. Although most of the patients had a leukocytosis and a few had severe anemia, the phenomenon also occurred in association with leukopenic states and in some instances in which the red blood cell counts were higher than normal. We found no relationship of this reaction to the level of blood nonprotein nitrogen, sugar, cholesterol, or protein. The sedimentation rates were high in the majority of the patients studied.

The test was performed in the same manner as a red blood cell count, using a red blood cell pipet and Hayem's solution as the diluent. In a positive reaction, agglutination occurred almost immediately and became quite pronounced within one-half hour. Clumps of brownish particles were seen floating in the fluid, which soon settled to the dependent portion of the pipet. Vigorous shaking caused no noticeable changes in the size of the clumps. Microscopic examination was not necessary, as the macroscopic reaction was sufficient for accurate interpretation. Under the microscope the clumps resembled "irregular entangled masses of red blood cells, some containing only a few cells, others a great many".²

Tests gradually became negative in patients who recovered, that is, agglutination did not occur, but continued positive in those who later died, indicating that while agglutination of erythrocytes is not necessarily a grave prognostic sign, it is a sign which throws light on the activity of the illness at the time the patient is tested. A number of patients with neoplasms continued to give positive reactions, even when retested after a period of six months.

In nineteen tests of bacterial pneumonias, one test from a seriously ill patient was negative throughout the course of the disease; sixteen tests were strongly

* Received for publication, January 9, 1947.

positive but became negative upon the patient's recovery; and two tests on patients who subsequently died showed clumping.

Agglutination did not occur in approximately two hundred apparently normal individuals and in a relatively few critically ill patients when first seen. The test is of value when it is positive and repeated periodically. Subsequent negative results usually are indicative of recovery.

Acknowledgment. The authors wish to thank Dr. Eugene De Angelis, Associate Professor of Pathology, West Virginia School of Medicine, Morgantown, West Virginia, for valuable suggestions rendered in the preparation of the manuscript.

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HUMAN SERUM AS ENRICHMENT FOR SUGARS IN CULTURES OF NEISSERIAE*

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The problem of obtaining clear-cut fermentation reactions with members of the Neisseria group has been considered by many investigators. The pathogenic neisseriae, at best, grow slowly in ordinary fermentation mediums. At the present time there are a number of substances used for the purpose of enriching fermentation sugars. Among these substances, ascitic fluid and human or horse serum have probably been used more frequently than hydrocele or pleural fluid. The latter fluids are not recommended by Trowbridge and McConkey.⁵ A number of laboratories use sterile human serum or plasma for enrichment purposes but its practical use in routine work has not been stressed.

Hitchens² introduced a semisolid medium to which 1 per cent horse serum was added. The value of horse serum has been questioned by Peizer,⁴ who found that when horse, beef or sheep serum was used as an enriching substance, the *Gonococcus* fermented maltose as well as dextrose, because of the presence of the enzyme maltase in these serums. Satisfactory fermentation results were obtained, however, when human, rabbit or guinea pig serum was used.

Lankford, Scott, Cox and Cooke³ have shown that the *Gonococcus* requires complex protein substances for propagation and Gould, Kane and Mueller¹ have discussed starch from the standpoint of its ability to neutralize the inhibiting effect associated with agar. Due to the availability of human serum and its stability in the refrigerator at a temperature of 50 C., it seemed of interest to ascertain the value of human serum as an enrichment substance when used alone and in combination with starch.

METHOD

All strains of neisseriae studied were planted on dextrose starch agar (Bacto), half strength, according to the formula described in the Difco Manual, seventh edition, p. 99, 1943.

The slants were incubated for forty-eight hours at 37 C. and the growth on each slant was then loosened by scraping with a sterile loop. The bacteria were transferred into sterile bottles containing a few glass beads and sterile saline which had previously been warmed to 37 C. The suspension of organisms in each bottle was then diluted to compare with a No. 2 nephelometer standard (six hundred million bacteria per cc.). The sugar inoculations were made from this suspension using a pipet delivering 1 drop (approximately 0.04 cc.) directly onto the surface of the sugar mediums.

Sugar base for the Neisseria group is prepared as follows: Dissolve 20 gm. of proteose peptone No. 3., 5 gm. of sodium chloride, 1 gm. of agar in 1 liter of distilled water by heating.

* Received for publication, March 24, 1947.

Cool, add 25 cc. of 0.04 per cent phenol red indicator and adjust the pH to between 7.4 and 7.6. Divide into three portions and prepare 1 per cent solutions of maltose, dextrose and saccharose. Autoclave at from ten to twelve pounds pressure for from ten to twelve minutes. Place in tubes, in 2 cc. quantities; add 0.1 cc. of a 20 per cent solution of sterile human serum in saline and mix.

TABLE 1

FERMENTATION OF DEXTROSE* CONTAINING VARYING QUANTITIES OF HUMAN SERUM BY SEVERAL STRAINS OF GONOCOCCI

Amount of human serum added.....	1%		2%		3%		4%		5%		0	
Length of incubation in hours.....	24	48	24	48	24	48	24	48	24	48	24	48
STRAIN OF GONOCOCCUS USED	DEGREE OF FERMENTATION†											
S-1	4	4	2	4	2	3	1	3	2	3	0	1
S-2	2	3	3	3	2	3	3	3	3	3	0	2
S-3	2	4	2	2	3	4	2	3	3	3	2	2
S-4	4	4	3	4	4	4	2	3	4	4	0	1

* Maltose and saccharose yielded no fermentation.

† Degree of fermentation: 0, none; 1, slight; 2, weak; 3, moderate; 4, strong.

TABLE 2

FERMENTATION OF DEXTROSE* WITH AND WITHOUT ENRICHING SUBSTANCES BY VARIOUS STRAINS OF GONOCOCCI

Strain of Gonococcus used.....	S4951			Sf			Sd			S1			S2			S3		
Length of incubation in days....	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4
ENRICHING SUBSTANCE† ADDED TO DEXTROSE MEDIUM	DEGREE OF FERMENTATION†																	
Serum plus starch.....	4	4	4	4	4	4	2	2	3	3	4	4	1	2	3	2	4	4
Serum.....	4	4	4	3	4	4	3	4	4	4	4	4	3	4	4	3	3	4
Starch.....	1	2	4	0	1	2	1	1	2	1	2	2	0	3	3	1	2	2
No enrichment.....	0	0	0	0	0	2	0	0	0	0	0	0	0	0	2	0	1	2

* Maltose and saccharose yielded no fermentation.

† Serum plus starch was used in the following concentration: soluble starch, 0.5 per cent; sterile human serum, 1.0 per cent.

‡ Degree of fermentation: 0, none; 1, slight; 2, weak; 3, moderate; 4, strong.

In order to determine an optimum concentration of sterile human serum for enrichment, varying quantities were tried with four strains of gonococci. As illustrated by Table 1, there seemed to be little difference in degree of fermentation with amounts varying from 1 to 5 per cent. In all of the following tests Seitz-filtered human serum was used with a final concentration of 1 per cent in the sugar tubes. No enrichment was added to the control sugar mediums.

Starch alone exhibited earlier fermentation than did the controls. In combinations with serum, however, reactions could be interpreted after a twenty-four hour period of incubation. With serum alone, the reactions were as good as, and in some instances better than, the starch-serum combination (see Tables 2 and 3).

SUMMARY

A concentration of 1 per cent sterile human serum in *Neisseria* fermentation mediums is a satisfactory enrichment substance. The growth in almost all cases was sufficient at the end of twenty-four hours to produce acid in the diag-

TABLE 3
FERMENTATION OF DEXTROSE AND MALTOSE,* WITH AND WITHOUT ENRICHING SUBSTANCES,
BY VARIOUS STRAINS OF MENINGOCOCCI

Strain of Meningococcus used ...	S2631						S4629						S1					
	Dextrose			Maltose			Dextrose			Maltose			Dextrose			Maltose		
Length of incubation in days. ...	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4
ENRICHING SUBSTANCES ADDED TO SUGAR MEDIUMS	DEGREE OF FERMENTATION†																	
Serum plus starch.	2	4	4	2	4	4	4	4	4	4	4	4	2	3	4	3	4	4
Serum.	1	3	4	2	3	4	4	4	4	3	4	4	3	4	4	3	4	4
Starch.	1	1	4	1	2	4	3	4	4	2	4	4	1	1	2	1	2	2
No enrichment.	1	1	4	0	1	4	1	2	2	1	4	4	0	0	1	0	0	0

* Saccharose yielded no fermentation.

† For explanation of symbols, see footnote to Table 2.

nostic sugars. In the control sugar mediums, it required more than twenty-four hours, and usually forty-eight hours, for reaction to take place. The use of starch as an enrichment is of questionable value.

Acknowledgment is made to James N. Patterson, M. D., and Morris S. Sommers, B. S., for their valuable assistance in the preparation of this manuscript.

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INTERFERENCE WITH SULFA DETERMINATION OF BLOOD BY *HEMOPHILUS INFLUENZAE**

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In a patient who was receiving sulfadiazine in the treatment of meningitis due to *Hemophilus influenzae*, extremely high blood sulfa values were obtained during the acute stage of the disease which seemed to be entirely out of proportion to the dose of the drug administered (Table 1). Sulfa determinations (Bratton

TABLE 1

SULFADIAZINE VALUES IN BLOOD OF PATIENT WITH MENINGITIS AND BACTEREMIA DUE TO
Hemophilus influenzae, TYPE B

DATE, 1946	BLOOD CULTURE	SPINAL FLUID CULTURE	BLOOD SULFADIAZINE LEVEL MG. PER 100 CC.
Feb. 13		<i>H. influenzae</i>	
20	<i>H. influenzae</i>	<i>H. influenzae</i>	86.9
25		<i>H. influenzae</i>	61.5
27		<i>H. influenzae</i>	43.4
March 4	No growth	<i>H. influenzae</i>	
9		No growth	18
16	No growth	No growth	5
April 2		No growth	

Highest daily dose received by patient was 5 gm. sodium sulfadiazine by clysis.

and Marshall's method) were, therefore, performed on filtrates from seventy-two hour hemopeptone and chocolate broth cultures of indole-positive *H. influenzae*, Type B, obtained from patients with meningitis. These 14 strains of *H. influenzae*, Type B, produced a substance that gave a colorimetric reaction with the reagents used in performing sulfa determinations. Equivalent colorimetric values ranging from 2.3 mg. to 6.5 mg. sulfadiazine per 100 cc. of blood were obtained (Table 2). Controls with filtrates of the culture mediums employed in growing the organisms (chocolate and hemopeptone broth) gave negative results.

I believed that the colorimetric reactions obtained from cultures of *H. influenzae* were due to the production of indole. To test the validity of this assumption, an aqueous solution containing 0.1 mg. of indole per 100 cc. was treated in the same way as blood in sulfa determinations. A color reaction was obtained which gave a colorimetric reading having the equivalent value of 4.6 mg. sulfadiazine per 100 cc. of solution.

* Received for publication, April 11, 1947.

TABLE 2
 COLORIMETRIC READINGS OF FILTRATES OF 72 HOUR CULTURES OF *H. influenzae*, TYPE B,
 IN EQUIVALENT VALUES OF MG. OF SULFADIAZINE PER 100 CC. OF BLOOD

NUMBER OF CULTURE	INDOLE	EQUIVALENT COLORIMETRIC VALUE IN MG. OF SULFADIAZINE PER 100 CC.
1	+	4.4
2	+	6.0
3	+	4.6
4	+	4.0
5	+	3.2
6	+	2.4
7	+	2.3
8	+	2.5
9	+	2.4
10	+	6.5
11	+	6.4
12	+	5.7
13	+	6.0
14	+	5.0
Sterile hemopeptone broth	—	0
Sterile chocolate broth	—	0

SUMMARY

Extremely high blood sulfa levels were obtained in a patient with meningitis and bacteremia due to *Hemophilus influenzae*. It was found that indole, which is formed by this organism, gives a color reaction similar to that given by sulfa drugs.

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HEMOLYTIC ANEMIA ASSOCIATED WITH MALIGNANT DISEASES*

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It has been appreciated for many years that on rare occasions hemolytic anemia may occur in patients with malignant diseases, such as Hodgkin's disease, chronic lymphatic leukemia, reticulo-endotheliosis, metastatic carcinoma, sarcoma of the spleen, myelogenous leukemia, lymphosarcoma, giant follicular lymphoblastoma and in patients with Boeck's sarcoid. The hope that such cases might provide important information regarding the etiology or pathogenesis of hemolytic states has not been fulfilled; it has been shown, however, that in such anemias, spherocytosis of the red blood cells may be a temporarily acquired property. In this paper we shall not consider the pathogenesis of the anemias that often accompany the above-mentioned diseases. They develop from diverse causes; excessive hemolysis may be a contributing factor.^{17, 18, 25} Our attention will be directed only to those cases with hemolysis so marked as to result in the hematologic picture of hemolytic anemia. Anemias of this type continue to be of extraordinary interest because they often pose difficult diagnostic problems. Morphologically and physiologically they may be identical with either the well-known familial hemolytic anemia or other hemolytic anemias of unknown causation occurring sporadically in adults. In four patients (Cases 1, 2, 3, 9), the diagnosis of both diseases was readily made; in two others (Cases 4, 8), there was, at first, little or no evidence of any disease other than anemia; in one patient (Case 10), after prolonged observation, symptoms and signs appeared which indicated the presence of another morbid process; in one patient (Case 6), splenectomy for hemolytic anemia revealed pathologic changes in the spleen which were wholly unexpected; in two patients (Cases 5, 7) failing to improve after splenectomy, thorough study yielded evidence of other diseases.

The infrequency of frank hemolytic anemia in these chronic diseases and the fact that attention is already directed toward the underlying lesion may render clinical detection of the type of anemia difficult. This is especially true when the anemia is not severe or when the other manifestations of hemolysis (reticulocytosis, bilirubinemia, spherocytosis) are intermittently absent or are overlooked. Hematologic study of such cases must be particularly careful.

There has been no actual proof that the hemolytic anemias which may complicate the diseases mentioned above are definitely related to these diseases but, because of their association, most clinicians believe that a close relationship does exist. Sometimes it is possible to show that the associated diseases are not related. Thus, recently in a patient with undoubted familial hemolytic anemia in whom anemia had been present for thirty years, an asymptomatic carcinoma

*Received for publication, May 29, 1947.

of the splenic flexure was found at the time of splenectomy. Also, it is well known that an individual may have the hereditary substrate of familial hemolytic anemia and never be aware of it. Such latent cases in which slight, persistent icterus, splenomegaly, anemia, reticulocytosis or spherocytosis are present may be difficult to interpret when complicated by the presence of other diseases. Case 1 illustrates the considerable doubt which may exist of the relationship between hemolytic anemia and another associated disease (Boeck's sarcoid).

CASE 1

Boeck's Sarcoid

G. O., a Negro female child, who was in The Mount Sinai Hospital in 1939 and 1946, was first found to have a severe hemolytic anemia without spherocytosis at the age of six months. After a remission of seven years, she had a recurrence of hemolytic anemia, but, on this occasion, with spherocytosis and Boeck's sarcoid.

This child was first hospitalized because of pallor and vomiting. Examination revealed the presence of florid rickets in addition to moderate enlargement of both the liver and spleen. Lymphadenopathy was not present. The pertinent laboratory findings* included hemoglobin, 38 per cent; erythrocytes, 2,500,000; leukocytes, 23,000; platelets, 130,000. The nucleated erythrocytes in the peripheral blood varied from 1 to 70 per 100 leukocytes and the reticulocytes ranged from 4.2 to 8.2 per cent. There were no spherocytes and malarial parasites were not present. The fragility test of erythrocytes to hypotonic saline revealed beginning hemolysis in 0.44 per cent sodium chloride and complete hemolysis in 0.28 per cent solution. The blood Wassermann and tuberculin patch tests were negative and the icterus index was 10. The bone marrow was normo-erythroblastic.* The child was treated with liver, iron and six blood transfusions. At the time of discharge the hemoglobin was

**Methods:* Hemoglobin determinations were performed visually by the acid hematin method (15.6 gm. per 100 cc. of blood = 100 per cent); platelet counts were performed in a manner similar to red blood cell counts except that 3.8 per cent sodium citrate was used as the diluent (normal 120,000 to 300,000 per cu. mm.); reticulocyte counts were done with brilliant cresyl blue by the dry method (normal values less than 2 per cent); erythrocyte fragility tests were done with hypotonic saline with red blood cells freed of plasma by washing with physiologic salt solution, and the results were observed visually after centrifugation.

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60 per cent. During this three-month period of hospitalization, she had a number of short periods of mild fever which coincided with sudden falls in hemoglobin. The liver and spleen remained unchanged. The patient was seen periodically thereafter and she developed normally. The liver and spleen receded and were no longer palpable. Without any special therapy she maintained a hemoglobin value of 75 per cent. Her diet was adequate but suboptimal.

At the age of eight years, six weeks before her second admission, she developed bilateral iridocyclitis. The lymph nodes were moderately enlarged. The lungs were clear. A hemic apical systolic cardiac murmur was present. The liver was palpable 2 cm. below the right costal margin and the spleen could be easily felt as a firm smooth organ 4 cm. below the left costal margin. Just mesial to the spleen a separate firm lobulated mass was palpated and regarded as enlarged retroperitoneal lymph nodes.

Examination of the blood revealed: hemoglobin, 43 per cent; erythrocytes, 3,060,000; leukocytes, 6600; platelets, 270,000; nonsegmented neutrophils, 6 per cent; segmented neutrophils, 49 per cent; monocytes, 8 per cent and lymphocytes, 37 per cent. The reticulocytes varied between 5.5 and 15 per cent. The mean corpuscular volume was 62 cubic microns. The stained erythrocytes appeared microcytic and hypochromic; spherocytes were present. The saline fragility test of the erythrocytes on two occasions revealed hemolysis starting in 0.56 per cent sodium chloride; complete hemolysis occurred once in 0.25 per cent solution and once in 0.20 per cent solution. "Sickling" of the red blood cells was absent. Examination of the sternal bone marrow obtained by aspiration revealed a cellular marrow containing 2.4 per cent erythroblasts and 60.8 per cent normoblasts. A biopsy of an axillary lymph node showed changes typical of Boeck's sarcoid. The Mantoux test was negative with 1 mg. of old tuberculin. The blood chemistry was normal. Extensive roentgenologic examination of the chest, bones and gastro-intestinal tract showed no abnormalities.

During the hospitalization of six weeks the patient was afebrile. Soon after admission she developed hydrarthrosis, first of one knee and then of the other. Aspiration of the joints revealed straw-colored fluid which was negative on culture and after guinea pig inoculation. Physical and blood examinations of the mother, father and four siblings were normal.

There was no evidence of a familial or hereditary basis for the hemolytic anemia in this patient. It seems very improbable that the hemolytic anemia at the age of six months can be related to the Boeck's sarcoid found seven and one-half years later. It is not entirely clear that any relationship exists at the present time between the anemia and the sarcoid. Future observations may provide more evidence on this point.

Singer and Dameshek²³ were the first to use the term "symptomatic hemolytic anemia". They defined it as a hemolytic syndrome which showed a definite etiologic relationship to underlying diseases, as we have outlined above. In addition, they mentioned its occurrence in liver disease and in various acute and chronic infectious conditions. We shall use this term throughout the present paper and shall draw attention to features of symptomatic hemolytic anemia that have not been adequately considered in the literature. We shall first discuss the findings in the blood, bone marrow and spleen, concluding with a consideration of therapy. The discussion is based on a review of the literature and on 10 cases personally studied which include 1 case of Boeck's sarcoid, 3 cases of chronic lymphatic leukemia, 3 cases of Hodgkin's disease, 1 of lymphosarcoma, 1 of giant follicular lymphoblastoma and 1 of colloid carcinoma.

FINDINGS IN PERIPHERAL BLOOD

The findings in the peripheral blood in cases of symptomatic hemolytic anemia may indicate the presence of the underlying condition, *e.g.*, leukemia, and may reveal the usual morphologic abnormalities of the hemolytic state. It is now agreed by most observers that *spherocytosis* of the erythrocytes is an abnormality that may occur in many different conditions characterized by excessive hemolysis. The importance of detecting this change in the red blood cells is that it indicates the presence of a hemolytic state. It indicates nothing, however, concerning the nature of the hemolysis. Spherocytosis has been described in about 50 per cent of the reported cases of symptomatic hemolytic anemia. It was present in 5 of the 10 cases reported here. We are in disagreement with the viewpoint expressed by Thompson²⁴ that spherocytic hemolytic jaundice is synonymous with congenital hemolytic jaundice. About one-half of the cases of symptomatic hemolytic anemia revealed decreased resistance of the erythrocytes to hemolysis by hypotonic salt solution.

The *anemia* is usually of rapid onset and the clinical picture that of a subacute hemolytic process. Moderate to very severe grades of anemia may be observed. The hemoglobin may be less than 20 per cent and the red blood cells less than 1,000,000 per cu. mm. The color index is usually 1.0 or a little higher, and values over 1.2 are uncommon. The determination of the size of the erythrocytes by corpuscular constants is not reliable in hemolytic anemia because of the usual presence of reticulocytes, which are larger than nonreticulated erythrocytes. Consequently, reports cannot be accepted that the mean corpuscular volume is markedly elevated and indicates macrocytosis. In such instances, measurement of the diameters of the red blood cells by the Price-Jones technic, separating reticulated from nonreticulated cells is required. We observed macrocytosis in 3 of the cases we studied. It was an infrequent finding in cases reported in the literature.

The *leukocyte count* is greatly influenced by the underlying disease and in certain cases any changes that might occur as a result of the hemolytic process are masked. This is likely to happen in cases of leukemia in which counts as high as 375,000 per cu. mm. are observed. Leukocyte counts of less than 4000 per cu. mm. have been observed in patients with giant follicular lymphoblastoma, metastatic carcinomatosis and Hodgkin's disease. Except for these, the usual finding is a moderate leukocytosis as in other hemolytic anemias.

The *differential blood count* in instances of leukemia with symptomatic hemolytic anemia may be characteristic of the former condition. There have been a few patients in whom leukemia could not be diagnosed before splenectomy but in whom leukemic changes became apparent¹¹ after this procedure. It is usual to observe a shift to the left in the differential count. Myelocytes are not present in large numbers, except after splenectomy. Myeloblasts are distinctly unusual except in instances of myeloid leukemia.

In our patients, *normoblastemia* was observed in 50 per cent, the highest count being 15 normoblasts per 100 leukocytes. In the literature, about one-half the cases were reported to show small numbers of these cells. There have

been several observations which indicate marked "blast" crises after splenectomy and up to 400 normoblasts per 100 leukocytes have been observed at such times. In unusual cases before splenectomy, up to 125 nucleated red blood cells per 100 white blood cells have been observed and megaloblasts as well as erythroblasts have been present.¹⁹

The *reticulocyte count* is a reliable indication of the severity of a hemolytic state, despite the fact that it is an indirect measure of hemolysis. The reticulocytosis obviously depends on the regenerative response of the bone marrow to hemolysis and a high reticulocytosis, in a general way, indicates an active normoblastic and erythroblastic hyperplasia of the marrow. Occasionally, however, severe hemolysis may occur without much reticulocytosis. The number of reticulocytes in our cases of symptomatic hemolytic anemia varied between 5 and 87 per cent and was of the same magnitude as observed in other severe hemolytic anemias.

There are relatively few *platelet counts* reported in the literature in symptomatic hemolytic anemia. In 2 of the 10 cases that we studied, there was a moderate thrombocytopenia and, in both, resulting hemorrhagic manifestations were apparent (Cases 3 and 4). Several similar cases, some with more marked evidence of bleeding, have been reported.^{1, 6, 7, 10, 15, 27} Among these there have been two instances of metastatic carcinoma involving the skeleton and others of lymphatic leukemia, Hodgkin's disease, myeloid leukemia, lymphosarcoma and reticulo-endotheliosis. Thrombocythemia has not been observed.

The above described findings of normoblastemia, myelocytes in the peripheral blood and thrombocytopenia, although not the rule in symptomatic hemolytic anemia, occur often enough to warrant discussion with relation to the differentiation between such cases and "leuko-erythroblastic" anemia as described by Vaughan²⁵ in skeletal carcinomatosis and osteosclerotic anemia. Leuko-erythroblastic anemia is characterized by a variable degree of anemia, in which the most striking finding is the large number of normoblasts and erythroblasts which are out of proportion to the severity of the anemia. In addition, there may be a small percentage of myelocytes and myeloblasts. Thrombocytopenia is a frequent finding and reticulocytosis up to 7 to 10 per cent may also be observed. It is immediately apparent that such changes are similar to those described in the blood picture in symptomatic hemolytic anemia. For differentiation, the degree of reticulocytosis in the latter is usually much more marked and is, in general, proportional to the severity of the hemolysis and anemia. The presence of myelocytes, normoblasts and erythroblasts in the peripheral blood is likewise dependent on the severity of the anemia and the regenerative response of the bone marrow. In leuko-erythroblastic anemia this situation does not prevail; the presence of immature myeloid and erythroid cells in the circulating blood is unrelated to the anemia and the disproportion between the large numbers of such cells and the relative mildness of the anemia may be striking. Reticulocytosis of marked degree is not apparent. While the percentage of nucleated red blood cells may be high and that of reticulocytes low in leuko-erythroblastic anemia, in symptomatic hemolytic anemia the reverse is almost always true.

Spherocytosis of the erythrocytes is a common finding in symptomatic hemolytic anemia, but has rarely been described in leuko-erythroblastosis. In the latter condition almost any other type of erythrocyte may be observed, *e.g.*, normocytic, macrocytic, hypochromic or elliptical. Finally, high fecal urobilinogen excretion² is found only in hemolytic states.

We have not found any indication in the literature or in the cases which we studied that *agglutinins* or *hemolysins* are responsible for the anemia in symptomatic hemolytic anemia which accompanies certain malignant diseases. None of these cases, however, was studied by the newer methods.^{3, 8} Haden¹¹ mentioned the presence of auto-agglutinins in a case of chronic lymphatic leukemia, but did not state the titer. In another case¹⁰ auto-agglutination may have been related to infectious mononucleosis and anti-O agglutinins. Neither hemoglobinuria nor hemoglobinemia has ever been described in the cases under discussion.

FINDINGS IN BONE MARROW

The morphologic alterations that occur in normal bone marrow in response to excessive hemolysis of erythrocytes are well known, and consist of *hyperplasia* of the cellular marrow and a quantitative change in the percentage of *erythrocytic elements*. The normoblasts and erythroblasts of the normal marrow may be increased from a normal value of 20 per cent to 40 per cent or more and, in rare instances, even up to 80 per cent. Some of the diseases in which, as we have seen, hemolytic anemia may be merely symptomatic are likewise accompanied by their own quantitative and qualitative alterations of bone marrow. The effect of two disease processes in the same marrow may need to be determined.

Our cases were repeatedly studied from this point of view by puncture of the sternum and examination of the aspirated material. In Case 10, in which the diagnosis was not certain, but appeared to be chronic lymphatic leukemia, the lymphatic infiltration was not evident at first when the hemolytic anemia was severe. This may have been due to the normoblastosis of 69 per cent. One month later, when the anemia had disappeared, the normo-erythroblasts were 14.4 per cent, whereas evidence of lymphatic infiltration was now present since the lymphocytes amounted to 20.8 per cent. In Case 9, with chronic lymphatic leukemia, two sternal punctures performed during the period of active hemolytic anemia revealed marked infiltration with lymphocytes (71.4 per cent and 36.4 per cent), while the normo-erythroblastic percentage in each instance was 19.4 per cent and 38.8 per cent. In this patient, both disease states contributed to the bone marrow picture. In Case 2, the normo-erythroblastosis of the bone marrow (56.2 per cent and 66 per cent) so overshadowed the lymphocytic infiltration of chronic lymphatic leukemia (the lymphocytes varied from 2.0 to 9.6 per cent) as to make the latter almost insignificant. By contrast, in the usual cases of chronic lymphatic leukemia without hemolytic anemia, the lymphocytes may vary from 50 to 90 per cent whereas the normo-erythroblasts may not exceed 2 per cent. In Case 3, in which the patient had giant follicular lymphoblastoma, the usual finding of that disorder, namely, an increase in the hematogones, was

not observed and this may have been due to the normo-erythroblastosis of 70 per cent. In Case 8, carcinoma cells could not be found in the bone puncture and the normo-erythroblastic increase amounted to about 50 per cent. The details of repeated bone marrow examinations are given in the following case.

CASE 2

Chronic Lymphatic Leukemia

W. H., a man 61 years old, had been well until three weeks before entering The Mount Sinai Hospital, when he suddenly became weak and fainted. He had not been exposed to toxic agents and neither the patient nor his relatives gave any history of anemia, jaundice or splenomegaly. Immediately before admission two blood transfusions (1000 cc.) were given. Examination revealed pallor and icterus; generalized lymphadenopathy was present. The liver was palpable 3 fingerbreadths below the right costal margin and the spleen 1 fingerbreadth below the left costal margin. A soft systolic murmur was audible over the apex of the heart.

The blood examination showed hemoglobin, 39 per cent; erythrocytes, 1,180,000; leukocytes, 125,000; platelets, 400,000.* There were 4 per cent nonsegmented neutrophilic leukocytes, 11 per cent segmented neutrophilic leukocytes, 84 per cent lymphocytes, 1 per cent neutrophilic myelocytes, 5 normoblasts per 100 leukocytes and 65 per cent reticulocytes. The erythrocyte fragility test showed hemolysis starting in 0.56 per cent sodium chloride and complete hemolysis in 0.28 per cent solution. There was moderate stippling of the erythrocytes which in stained smears were either microcytic (spherocytes) or macrocytic (reticulocytes). Plasma hemoglobin was less than 5 mg. per cent; icterus index, 18 to 62; van den Bergh, slightly delayed positive; cold hemagglutinin titer, 1/40; heat resistance test of erythrocytes, negative; acid hemolysis test (Ham), negative; and hemagglutinins (37 C.) and hemolysins (homologous and heterologous group O erythrocytes), negative. The results of three sternal bone marrow aspirations are given in Table 1. Chemical analysis of the blood revealed normal findings except that the cephalin-cholesterol flocculation test was 2 to 4 plus. The blood Wassermann test was negative. In numerous urinalyses the only abnormality was a urobilinogen value (Wallace-Diamond) varying between 1/10 and 1/100. Biopsy of an axillary lymph node revealed a marked infiltration by lymphocytes compatible with chronic lymphatic leukemia. The basal metabolic rate was plus 18 per cent. Roentgenologic examinations of the chest, skull and long bones did not reveal any abnormalities.

This patient was discharged after nine weeks because he refused to submit to splenectomy. Ten blood transfusions (5000 cc.) were administered during this period without reactions. The jaundice persisted but the temperature was never elevated. Repeated reticulocyte determinations were recorded as 60, 28, 24, 22 and 9 per cent. Prior to discharge, the hemoglobin was 66 per cent; the leukocyte count, 22,400; the segmented neutrophils, 25 per cent; the eosinophilic leukocytes, 2 per cent; the lymphocytes, 70 per cent; and the monocytes, 3 per cent. There was no change in the lymphadenopathy, splenomegaly or hepatomegaly. The patient died at home soon thereafter and no autopsy was performed.

The features of acute spherocytic hemolytic anemia were clear-cut in this patient. The absolute and relative lymphocytosis of the peripheral blood, generalized lymphadenopathy and the lymphocytic infiltration of a lymph node indicated the presence of chronic lymphatic leukemia. The absence of lymphocytic infiltration in the bone marrow might have been due to the intense normo-erythroblastic hyperplasia resulting from the hemolytic process.

* For method used see footnote to Case 1.

Analysis of the differential counts of sternal bone marrow spreads indicates that the *excessive normo-erythroblastosis* of hemolytic anemia may mask the presence of an underlying disease state that otherwise could be diagnosed by this examination. At the same time, the presence of an underlying disorder which produces characteristic changes in the myelogram may partly inhibit the normo-erythroblastosis of superimposed hemolytic anemia.

Many of the bone marrow studies reported in the literature were made on sections of material obtained at autopsy and the results are not reported in sufficient detail. The findings in general conform to our own. In Davis'⁷ fourth case of myeloblastic leukemia there was scant normoblastic erythropoiesis, most of the cells in the marrow being promyelocytes and myeloblasts. Despite the apparent inhibition of erythropoiesis, the reticulocytes in the blood numbered 66 per cent. In Feldman's¹⁰ case of subacute lymphatic leukemia, there was a progressive increase in the number of lymphocytes at the expense of erythro-

TABLE 1
PERCENTAGES OF NUCLATED CELLS IN STERNAL BONE MARROW IN CASE 2*

	TOTAL NUCLATED CELLS (PER CU. MM.)	NEUTROPHILS			LYMPHO- CYTES	HEMATO- GONES	ERYTHRO- BLASTS	NORMO- BLASTS
		Myelo- cytes	Nonseg- mented	Seg- mented				
On admission.....	300,000	5.0	7.6	3.0	2.0	12.3	10.0	54.6
4 weeks later.....	625,000	8.0	11.0	4.0	8.6	8.0	11.0	45.2
8 weeks later.....	875,000	10.4	7.2	2.8	9.6	0.4	14.8	51.2

* In all instances in this paper only the significant cells are classified.

poietic forms, and in Singer and Dameshek's²³ cases 4 and 5 of chronic lymphatic leukemia the marrow was greatly infiltrated with lymphocytes.

PATHOLOGIC CHANGES IN SPLEEN

One of the more interesting phases of the pathogenesis of symptomatic hemolytic anemia concerns the morphologic changes in the spleen. Our material and the cases reported in the literature indicate that "*arterial*" *hyperemia* of the spleen (also described as active hyperemia, overfilling of the pulp with blood or collapsed venous sinusoids in a lake of blood), as seen invariably in congenital hemolytic anemia, occurs also in symptomatic hemolytic anemia.^{1, 2, 4, 14, 20, 27, 28} We have observed such a finding in Hodgkin's disease (Case 5), lymphosarcoma (Case 7) and metastatic carcinoma (Case 8). Furthermore, we, and others,¹² have observed the same pathologic picture in the spleen in hemolytic anemia unassociated with other diseases in middle-aged or elderly persons in whom there was no reasonable evidence of a congenital or familial origin of the anemia. In the few cases of secondary hemolytic anemia associated with carcinoma reported in the literature,^{2, 7, 27} metastases were not present in the spleen, although they were widespread, particularly involving the bone marrow. A possible exception is the case of Faulds and Mackay.⁵ In most of these cases, the spleen

was enlarged and, as far as one can ascertain, "active" hyperemia was the usual finding. Many of the cases of symptomatic hemolytic anemia with "arterial" hyperemia of the spleen reported in the literature^{1, 2, 4, 10, 20, 27} showed spherocytosis of the red cells of the circulating blood (Cases 5 and 8). There does not, however, appear to be an obligate relationship. In Case 7, "arterial" hyperemia of the spleen was present in a patient with macrocytic (nonspherocytic) erythrocytes. The occurrence of spherocytosis in the absence of "arterial" hyperemia of the spleen has been described.^{5, 6, 11, 13, 15, 23}

In addition, there are numerous reports in which the clinical and hematologic pictures in symptomatic hemolytic anemia were similar to or identical to those in the foregoing cases, but in which the spleen did not show "arterial" hyperemia. In some of these cases, spherocytosis of the red blood cells was observed, while in others, the erythrocytes were normal in size or were macrocytic. In such instances, the spleen often was involved by the underlying disorder, as by Hodgkin's disease, lymphosarcoma, chronic lymphatic leukemia.^{5, 7, 11, 13, 22, 23, 24, 29} In other cases erythrophagocytosis, myeloid metaplasia, reticulum cell hyperplasia and hemosiderosis were prominent findings.^{5, 14, 15}

PROBLEMS IN THERAPY

In considering therapy for this group of patients with symptomatic hemolytic anemia, several questions arise. Should the first attempt in therapy be to treat or eradicate the underlying disease? Should initial therapy be directed toward immediate alleviation of the excessive hemolysis? Is there any likelihood that the hemolytic process may subside spontaneously or, once established, is it likely to continue or become more severe?

The following remarks on radiotherapy are based on observations in our cases. The underlying disease was treated in only two of our patients, one having giant follicular lymphoblastoma and the other having Hodgkin's disease. In the former patient (Case 3), a complete course of radiotherapy could not be administered because of the development of severe leukopenia resulting from external irradiation to the spleen. In this patient there was no evidence of recession of the hemolytic activity, despite the fact that the spleen diminished markedly in size. The patient with Hodgkin's disease (Case 4) had only a moderately severe anemia and the degree of reticulocytosis was not striking. The hemolytic anemia did not appear to be the important part of the clinical picture. At the same time, the diagnosis of Hodgkin's disease could not be made until the disease had entered its terminal phase and the patient exhibited high fever. In this patient radiotherapy directed to the spleen, not only had no inhibitory effect on the size of this organ which continued to enlarge, but the moderate degree of anemia continued unchanged. The details of these cases follow.

CASE 3

Giant Follicular Lymphoblastoma

M. L., a 65 year old man, was under observation for two months. A diagnosis of hemolytic anemia was made on the basis of peripheral blood and bone marrow findings and a diagnosis of giant follicular lymphoblastoma was based on biopsy of an axillary lymph node.

Three months before admission, he noted the sudden onset of weakness and dyspnea. His hemoglobin was 32 per cent and was unaffected by adequate therapy with refined liver extracts. Two days before admission, purpura and edema of the legs were noted.

The relevant facts in the past history included the sudden onset of anemia without jaundice and a hemoglobin of 19 per cent six years previously. Splenomegaly was observed but the cause of the anemia was not ascertained. Treatment with transfusions and liver extract effected a partial remission. Five and one-half years ago he sustained a cerebral accident. Thereafter he was well until the onset of the present complaints. There was no family history of anemia, splenomegaly or jaundice.

The patient was well developed and nourished but somewhat pale and icteric. There was some puffiness of the lower eyelids and chemosis of the conjunctivae. There were numerous firm, discrete, movable lymph nodes. The heart and lungs were not remarkable. The liver and spleen were both greatly enlarged; the former was firm and its edge was felt 4 cm. below the right costal margin, while the spleen could be felt as low as the right lower abdominal quadrant. Slight pitting edema and numerous purpuric areas were noted over both legs.

Hematologic examination revealed the following: hemoglobin, 49 per cent; erythrocytes, 2,900,000; leukocytes, 3400; and platelets, 100,000. The segmented neutrophils numbered 46 per cent; nonsegmented neutrophils, 9 per cent; lymphocytes, 36 per cent; monocytes, 9 per cent; and reticulocytes, 8.5 per cent. The erythrocytes were of normal diameter on the stained slide and spherocytes were not observed. The saline fragility test of the erythrocytes revealed hemolysis starting in 0.44 per cent sodium chloride and complete hemolysis in 0.21 per cent solution. The bleeding time was one and one-half minutes, the coagulation time of venous blood was six minutes and the clot retracted normally. The capillary resistance test was negative. Study of the bone marrow obtained by sternal marrow aspiration revealed a total nucleated cell count of 160,000 per cu. mm. with 66 megakaryocytes per cu. mm. The neutrophilic myelocytes were 13 per cent; nonsegmented neutrophils, 10.0 per cent; segmented neutrophils, 1.4 per cent; lymphocytes, 1.4 per cent; hematogones, 2.7 per cent; erythroblasts, 7.3 per cent; normoblasts, 62.3 per cent.

The icterus index was 21, later falling to 12 and the direct van den Bergh reaction was negative; otherwise the blood chemistry was normal. Uric acid crystalluria was frequent. Bilirubinuria was absent and the urobilinogen was markedly elevated being positive in a 1/1280 dilution (Wallace-Diamond technic). Occult blood was not found in the feces. The blood Wassermann test was negative. Roentgenologic examination failed to disclose abnormalities in the lungs, mediastinum or gastro-intestinal tract. Esophageal varices were not present. An axillary lymph node biopsy revealed the typical changes of giant follicular lymphoblastoma.

The patient exhibited a low grade fever during the period of observation. Three treatments with x-irradiation of 50 r each were given to the four abdominal quadrants both anteriorly and posteriorly. There was no effect on the severity of the anemia but the spleen receded greatly in size. A fall in the white blood cell count to 1650 forced cessation of this therapy. He subsequently received five blood transfusions totaling 2500 cc., two of which were followed by mild pyrogenic reactions. Despite this, the hemoglobin at the time of discharge from the hospital was 38 per cent. The leukocyte count had risen to the level existing prior to radiotherapy. A complication of the leukopenia was a submandibular abscess which was incised. The wound healed rapidly as the leukocyte level improved. The edema and purpura cleared completely.

In this case it is probable that the giant follicular lymphoblastoma had been present for six years. Its natural history was punctuated by episodes of anemia, one at least being of the hemolytic type. The subsidence of the anemia which occurred early in the disease may have been due to a spontaneous remission of a secondary hemolytic anemia.

CASE 4

Hodgkin's Disease

S. J., 60 years old, a building superintendent, was studied extensively in the hospital during a two month period. The diagnosis of hemolytic anemia was readily made, but that of Hodgkin's disease was not made until after six weeks when lymphadenopathy appeared and biopsy was performed. His illness started four months before admission with weakness and dizziness. He lost 35 pounds in weight prior to admission. He was carefully examined repeatedly at another hospital but a definite diagnosis was not possible. At that time, after one month of illness, enlargement of the liver and spleen was found.

On examination he appeared pallid, subicteric and chronically ill. The lymph nodes were not enlarged. There were no abnormal findings in the heart or lungs. The abdomen was somewhat distended by the liver and spleen, the former extending 4 cm. below the right costal margin and the latter 5 cm. below the left costal margin.

Hematologic studies of the peripheral blood showed: hemoglobin, 56 per cent; erythrocytes, 2,550,000; leukocytes, 5700; platelets, 100,000; hematocrit, 26 per cent; mean corpuscular volume, 104 cubic microns. There was a mild macrocytosis and spherocytes were absent. The segmented neutrophils numbered 39 per cent; nonsegmented neutrophils, 17 per cent; segmented eosinophils, 6 per cent; lymphocytes, 30 per cent; monocytes, 8 per cent. The reticulocyte count was 12 per cent. The hypotonic saline fragility test of the erythrocytes revealed beginning hemolysis in 0.44 per cent sodium chloride and complete hemolysis in 0.24 per cent solution. The acid hemolysis (Ham) test was negative. Examination of the aspirated sternal bone marrow revealed a total nucleated count of 150,000 per cu. mm. with 88 megakaryocytes per cu. mm. The neutrophilic myelocytes were 15 per cent; eosinophilic myelocytes, 2 per cent; nonsegmented neutrophils, 10 per cent; segmented neutrophils, 4.4 per cent; segmented eosinophils, 3.0 per cent; lymphocytes, 8.3 per cent; erythroblasts, 9.7 per cent; normoblasts, 44.3 per cent; megaloblasts, 2.0 per cent. Blood chemistry was normal except for the icterus index which was 7. Radiographic examinations of the gastro-intestinal tract, chest, skull and long bones failed to disclose any pathologic lesion except for a pressure defect on the greater curvature of the stomach due to the enlarged spleen. The blood Wassermann test was negative and the basal metabolic rate, plus 16 per cent. On repeated urinalyses there was a small amount of albumin; the urobilinogen was positive up to dilutions of 1/40 (Wallace-Diamond test) and moderate numbers of granular casts and occasional red blood cells and white blood cells were apparent in the sediment. Fractional gastric analysis, following the administration of histamine, yielded normal amounts of free hydrochloric acid. The Mantoux test was negative with 1 mg. of old tuberculin.

During the first five weeks of hospitalization, the temperature fluctuated between 99 and 101 F. Thirty mg. per day of folic acid was administered orally, followed by large amounts of both crude and refined liver extract parenterally without any significant effect on the clinical picture. Frequent repetition of the blood examinations showed relatively little fall in the hemoglobin and red blood cells. The slight macrocytosis, elevation of the color index and icterus index and reticulocytosis persisted. At this time, lymphadenopathy of mild degree first appeared and biopsy of an axillary node showed Hodgkin's disease. Aspiration of the enlarged spleen yielded a small fragment of tissue showing fibrosis and very occasional abnormal reticulum cells.

By the time this definite diagnosis was made, the patient was entering the terminal phase of his illness. The temperature gradually rose to 105 F. The patient was given seven blood transfusions, totaling 3500 cc., without reactions, but a rise in the blood count could not be effected. Seven x-ray treatments of 75 r each were given over the spleen. Nevertheless, there was progressive enlargement of the spleen as well as of the liver and lymph nodes. At the time of exitus the spleen extended into the pelvis. Shortly before death, infiltrations and hemorrhages appeared in the ocular fundi and there was bleeding from the gums. At this period, the platelet count fell to 50,000 per cu. mm. The last few days were

marked by great debility, cough, cyanosis, bubbling râles in both lungs, severe icterus, bilirubinuria, oliguria and azotemia. An autopsy examination was not granted.

This was a case of Hodgkin's disease of six months' duration, during the course of which a moderately severe hemolytic anemia was observed. Clinically, the impression was gained that the blood dyscrasia did not contribute greatly to the short and rapidly fatal course. There was no clinical evidence of direct involvement of the bone marrow by Hodgkin's tissue.

Our other observations relevant to radiotherapy are derived from a patient with Hodgkin's disease (Case 5). This patient received systemic external radiation after splenectomy failed to control the excessive hemolytic tendency. In this instance, there was a most remarkable course over a five year period after splenectomy, when, on several occasions, radiotherapy to the neck or mediastinum effected dramatic remission in the hemolytic anemia.

Further information concerning the effect of radiation in symptomatic hemolytic anemia can be obtained from case reports in the literature on the treatment of lymphosarcoma, Hodgkin's disease or lymphatic leukemia with x-rays. In practically all these instances the details of the therapy and the time relationships between it and the anemia are not clearly stated. In two cases, one of chronic lymphatic leukemia²³ treated by the spray technic and directly over the spleen, and another of Hodgkin's disease in which treatment was applied to the cervical lymph nodes²² a symptomatic hemolytic anemia appeared so soon after radiation as to raise the possibility that the therapy might have been a factor in precipitating the hemolytic phase. In the former case, continuation of radiotherapy appeared to aggravate the hemolytic anemia. In four other instances^{7, 15, 24, 25} a similar sequence occurred but the interval between treatment and the anemia was so great as to indicate the probability of coincidence. In three reported cases, radiotherapy given after a diagnosis of symptomatic hemolytic anemia had been established seemed to have a beneficial effect on the anemia, but the results were not always striking. In one detailed case of probable chronic lymphatic leukemia,¹⁵ x-ray treatment over the spleen was followed by a rise in hemoglobin from 30 to 90 per cent, a rise in red blood cells from 1,260,000 to 4,300,000 per cu. mm., a fall in reticulocytes from 35 to 6.4 per cent and a disappearance of jaundice. In another incompletely described case of lymphatic leukemia by the same author, marked improvement hematologically and clinically followed similar therapy. In a case of lymphosarcoma,¹⁵ the marked improvement of the anemia following splenectomy and radiotherapy was probably due to splenectomy. In Haden's¹¹ case of possible sarcoid of the lung, an almost complete remission of a moderately severe hemolytic anemia was induced by radiotherapy to the pulmonary lesions. A probable mild spherocytic hemolytic anemia in a patient with chronic myeloid leukemia was improved by radiotherapy to the spleen in one of the cases reported by Paschkis.²¹

It is not possible at the present time to assess the value of radiotherapy in the treatment of the anemia in those cases of symptomatic hemolytic anemia associated with diseases otherwise sensitive to roentgen therapy. In view of the fact that the spleen in such cases may not be involved by lymphoblastoma^{4, 10,}

15, 20, 25, 29 and may reflect either the hemolytic tendency (*i.e.*, "arterial" hyperemia) or other changes, radiotherapy may or may not be of value if centered only over this organ. On the other hand, observations are too meager to state the probable effect of such treatment directed to the spleen in a patient whose spleen does not show the characteristic lesions of the underlying disease.^{5, 7, 11, 13, 22, 23, 24} There is also no available information concerning the effect of x-ray treatment in these cases when such treatment is directed to mediastinal or cervical regions prior to splenectomy. There are a few observations, including one thoroughly documented case that we have observed (Case 5), to indicate that after splenectomy such treatment may have a decidedly beneficial action. The possibility that x-ray treatment may be a precipitating factor in the hemolytic syndrome is of theoretical interest only, for even if such an eventuality occurs, it is certainly rare. Because of such variations in the localization of the pathologic changes and responses to radiation, it is not possible to predict the therapeutic effect of radiation in symptomatic hemolytic anemia secondary to the lymphoblastomas and leukemias. Most of the evidence indicates that, wherever possible, radiotherapy should be given a trial.

VALUE OF SPLENECTOMY

Our data provide conflicting facts regarding the value of splenectomy. In a patient with Hodgkin's disease (Case 5) the severe anemia, reticulocytosis, spherocytosis and increased fragility of the erythrocytes remained unchanged throughout the month following splenectomy and, as previously mentioned, was remarkably improved by radiotherapy. In another patient (Case 8) whose anemia was complicated by the presence of three diseases (ulcerative colitis, hematogenous tuberculosis and carcinomatosis), there was a temporary improvement after splenectomy. The red blood cell count rose from less than 1,000,000 per cu. mm. before splenectomy to more than 3,000,000 four weeks later and was accompanied by a fall in reticulocytes from 40 per cent to 4 per cent. This improvement was short-lived, however, for a severe relapse set in soon thereafter and continued for as long as the patient remained under observation. Similarly, in a patient with lymphosarcoma without spherocytosis (Case 7), there was temporary reduction in the severity of the hemolytic anemia after splenectomy. A severe relapse terminated in death. A splenectomy was also performed (Case 6) in a patient with Hodgkin's disease who had neither spherocytosis nor increased fragility of the erythrocytes. The response of the hemolytic anemia was most gratifying and persisted until the patient succumbed to her underlying disease. In this patient, the erythrocyte count rose from 2,300,000 to 4,650,000 per cu. mm. while at the same time the reticulocytes fell from 19 to 1 per cent. The following cases illustrate the variable effect of splenectomy on the hemolytic syndrome.

CASE 5

Hodgkin's Disease

A. M., a 33 year old woman, was under observation for twelve years. During this period she had severe hemolytic anemia for which a splenectomy was performed. She later received repeated courses of roentgen therapy for Hodgkin's disease, and finally died with a

picture of nontropical sprue due to amyloidosis of the small intestine. When first observed she stated that she had been anemic for many years, although study of the blood had never been made. There was no familial history of anemia, jaundice or splenomegaly and the patient had not been exposed to noxious agents. Marked weakness of one week's duration was the presenting symptom. She was icteric and pale and numerous hemorrhages were observed in the fundi. A few small anterior cervical lymph nodes were present. The liver was felt 1 fingerbreadth below the right costal margin but the spleen could not be palpated.

The results of blood studies on this and later occasions are given in Table 2. The differential blood count showed nonsegmented neutrophils, 15 per cent; segmented neutrophils, 64 per cent; segmented eosinophils, 2 per cent; lymphocytes, 15 per cent; monocytes, 4 per cent. The Wassermann test was negative; the icterus index was 17; the van den Bergh reaction was delayed positive and repeated urinalyses were negative. The total twenty-four hour urobilin excretion in the urine was 7.5 mg. There were no significant elevations of temperature. Because of the failure to note hematologic or clinical improvement, splenectomy was performed two and one-half weeks after admission.

The spleen was free of adhesions and was removed easily. There were no enlarged nodes at the hilum; there were no accessory spleens. The organ was somewhat enlarged and engorged with blood showing, on microscopic examination, marked hyperemia of the pulp and relatively empty collapsed sinusoids. The picture was that of "active hyperemia" as observed in hemolytic icterus. There was no evidence of Hodgkin's disease.

During the first month after splenectomy she exhibited an irregular temperature reaching as high as 102 F. The wound from the splenectomy healed promptly. She continued to show manifestations of hemolytic anemia. At this time she began to complain of pain in the right lower posterior portion of the chest; dullness and a pleural friction rub were detected. Roentgenographic examination revealed a homogeneous density in the region of the right lower lobe and an unusual enlargement of the tracheobronchial lymph nodes at the root of the right lung. Examination of the chest fluid for tubercle bacilli or other bacteria was negative. Because of the possibility that the thoracic lesion was due to a malignant process and also for diagnostic purposes, radiation therapy directed toward the mediastinum and the right lower lobe was given for two weeks. Considerable improvement resulted. The temperature fell to normal, the anemia improved and the spherocytosis and reticulocytosis diminished greatly. X-ray examination now showed complete resolution of the pulmonary process and distinct shrinkage of the lymph nodes. The patient was discharged from the hospital at this time and was followed in the clinic. The rapid subsidence of the lymphadenopathy suggested the presence of a malignant lymphadenoma.

The patient remained relatively well for four and one-half years at the end of which time she suddenly experienced a severe sore throat and observed enlarged cervical lymph nodes. The only therapy during this interval consisted of inorganic iron orally because of a tendency to develop hypochromic anemia. There was no definite evidence of hemolytic anemia over these years, although our observations are not complete regarding this point.

On this occasion, December 7, 1936, she was pale, but not icteric. There were many enlarged lymph nodes. X-ray examination of the chest revealed a large mass of lymph nodes in the superior mediastinum and at the root of the right lung. The hematologic studies are given in Table 2. The icterus index was 6. Repeated urinalyses indicated the presence of urobilinogen (Wallace-Diamond) in a dilution of 1/80. Biopsy of an enlarged supraclavicular lymph node revealed Hodgkin's disease. The patient was afebrile throughout her hospital stay of two and one-half weeks. X-ray treatment was applied to the mediastinum and the neck following which she improved greatly. She remained well enough to forego medical attention for ten months during which time she was not under supervision and did not take any medication.

She was observed again on October 18, 1937, complaining of pallor and weakness, and was found to be pregnant. There was no evidence of recrudescence of either the Hodgkin's disease or the hemolytic anemia; blood study (see Table 2) revealed a moderately severe

hypochromic anemia of pregnancy which improved considerably after iron therapy. Four months later she had an uneventful delivery of a living child.

Study was again interrupted by a period of two years during which time her general condition was good. Progressive enlargement of her cervical lymph nodes, requiring radiation treatment, brought her under our care again on February 26, 1940. For the first time, now, the hypotonic saline fragility test of her erythrocytes showed no increase and spherocytes were absent. Examination of the sternal bone marrow obtained by aspiration revealed erythroblasts, 1.0 per cent and normoblasts, 11.5 per cent.

She again experienced a prolonged remission free of complaints, which was followed by reappearance of the cervical and axillary adenopathy on April 12, 1944. Hematologic study showed mild hypochromic anemia with a complete absence of any of the signs of hemolytic anemia. Examination of the erythrocytes in the stained smear revealed a tendency to an increase in diameter, hypochromia and many target cells. The erythrocytes were thin; the mean corpuscular volume was 91 cubic microns. The icterus index was 3 and the acid hemolysis (Ham) and heat resistance tests were negative. Cold and warm (37 C.) agglutinins or hemolysins (homologous and heterologous group A) were not present.

A month after the cessation of x-ray treatment her terminal illness had its inception with severe unremitting diarrhea and anorexia. The stools were light brown, greasy and malodorous but free of blood. There was a 30 pound loss in weight. She was admitted to the hospital twice (August and October, 1944) and presented the picture of marked emaciation. There was no evidence of a hemolytic process. Treatment during this episode consisted of several blood transfusions and massive intravenous medication with vitamins, fluids, amino acids, glucose and sodium chloride. There was a temporary improvement followed by a fatal recurrence.

Autopsy examination revealed Hodgkin's disease, involving the lungs and the thoracic and retroperitoneal lymph nodes with fibrosis alternating with extensive caseation necrosis. The mesenteric nodes were not particularly involved. Focal amyloidosis of the submucosal vessels of the small intestines was responsible for the diarrhea. There were no abnormalities in the lumbar vertebral bone marrow. Several calcified calculi were present in the gallbladder. There were no accessory spleens nor was there any abnormality of the hemolymph nodes.

One cannot state with certainty that this patient did not have congenital hemolytic anemia complicated by, and unrelated to, Hodgkin's disease. It is not possible to evaluate her statement that she had been anemic for many years. We believe that the spherocytic hemolytic anemia in this case was causally related to the Hodgkin's disease for the following reasons: (1) Both diseases seemed to start at about the same time, clinically. (2) During a postoperative (splenectomy) period of one month there was no recession of hemolysis. (3) The excessive hemolysis, anemia, reticulocytosis and spherocytosis varied directly over part of the course with the activity of the Hodgkin's disease, diminishing or disappearing following x-ray radiation and reappearing with recrudescence of the lymphadenopathy. (4) The replacement and complete disappearance of the spherocytosis and the increased hypotonic saline fragility of the erythrocytes (after a period of eight years following splenectomy) by macrocytosis and target red blood cells.

CASE 6

Hodgkin's Disease

R. K., a 43 year old woman, had four admissions to the hospital, between January, 1943 and February, 1944. She was observed prior to development of anemia and during a phase

of moderately severe hemolytic anemia which was terminated by splenectomy. In her final illness, when evidence of excessive hemolysis was no longer present, she presented the clinical picture of rapidly progressive Hodgkin's disease. The patient and her family gave no

TABLE 2
HEMATOLOGIC FINDINGS IN CASE 5

DATE	Hb. PER CENT	ERYTHROCYTES MILLIONS PER CU. MM.	LEUKOCYTES PER CU. MM.	PLATELETS PER CU. MM.	HYPOTONIC SALINE FRAGILITY OF ERYTHROCYTES		SHAPE OF ERYTHROCYTES	RETICULOCYTES PER CENT	NUCLEATED RED CELLS PER 100 LEUKOCYTES
					Begin- ning	Com- plete			
Feb. 27, 1932	26 (1 T.)	1.12	13,050		0.60%	0.36%	Spherocytes 4+	57	9
Mar. 30	20 (3 T.)	1.28	12,000	55,000			Spherocytes 4+	58	15
Apr. 11	28	1.50	15,250	360,000			Spherocytes 4+	9	2
Splenectomy									
Apr. 16	(2 T.)								
Apr. 25	17				0.61%	0.32%	Spherocytes 4+	21	
May 3	19							44	
May 12	21	1.00	36,000	560,000			Spherocytes 4+	63	300
Radiotherapy to Mediastinum									
June 1	47	2.52	6,000	340,000	0.52%	0.28%	Spherocytes 2+	26	
June 7	53	2.31	7,000	360,000	0.52%	0.28%	Spherocytes 2+	10	
July 29	81	4.20	7,600	680,000				2.3	
May 1, 1933	72	4.75	9,200	360,000					
Mar. 5, 1934	63								
Mar. 29, 1935	60	3.9	13,000	280,000					
Nov. 27, 1936	43	2.6	12,200	400,000					
Radiotherapy to Neck and Mediastinum									
Dec. 12	(1 T.)				0.64%	0.32%	Spherocytes 4+	10	
Dec. 19	35	1.70	12,800	380,000				32	1
Dec. 25	(2 T.)								
Radiotherapy to Neck and Mediastinum									
Oct. 18, 1937	45	3.42	10,200	280,000			Spherocytes ?		
Feb. 4, 1938	60	4.75	18,200	320,000					
Pregnancy and Delivery of Living Baby									
Feb. 26, 1940	58	3.20	10,250	270,000	Normal		Macrocytes Target cells	0.5	1
Radiotherapy to Neck									
July 3	65 (1 T.)								

TABLE 2—Continued

DATE	HB. PER CENT	ERYTH- RO- CYTES MIL- LIONS PER CU. MM.	LEUKO- CYTES PER CU. MM.	PLATE- LETS PER CU. MM.	HYPOTONIC SALINE FRAGILITY OF ERYTHROCYTES		SHAPE OF ERYTHRO- CYTES	RETIC- ULO- CYTES PER CENT	NUCLE- ATED RED CELLS PER 100 LEUKO- CYTES
					Begin- ning	Com- plete			
Radiation Castration									
Nov. 25	50	3.58	14,000						
Dec. 20	70		6,900						
Apr. 12, 1944	64	3.76	9,750	340,000	0.44%	0.12%	Macrocytes Target cells	0.5	
Radiotherapy to Neck and Axilla									
Aug. 11	70	3.70	6,500						
Oct. 5	70		10,700						

T. indicates number of blood transfusions given, each of 500 cc.

history of jaundice, anemia or splenomegaly. She had not been exposed to any known noxious agent.

Her illness started in July, 1942 with menometrorrhagia followed soon thereafter by weakness and dizziness. Two blood transfusions were administered because the hemoglobin was found to be 50 per cent. Soon after she developed a generalized pruritus and she was admitted to the hospital. Examination revealed a well nourished patient. There were several small discrete lymph nodes in the posterior cervical regions and in both axillae. The lungs were clear and the heart was normal. The liver was palpable 1 fingerbreadth and the spleen 2 fingerbreadths below the respective costal margins. There was fine desquamation of the entire skin. She had no fever.

The blood counts and the results of biopsies are given in Table 3. The platelets numbered 140,000. The differential leukocyte count revealed segmented neutrophils, 62 per cent; nonsegmented neutrophils, 6 per cent; lymphocytes, 13 per cent; monocytes, 4 per cent; eosinophils, 15 per cent. Urinalysis yielded normal findings. Free hydrochloric acid was present in the gastric contents. Blood chemistry was normal except for proteins; and the Wassermann test was negative. Repeated determinations revealed the total serum protein to be 4.6, 5.8 and 5.3 gm. per cent and the globulin fraction 0.8, 0.9 and 0.5 gm. per cent. A diagnosis could not be made and the patient was discharged.

For the ensuing two months the patient was relatively well except for pruritus. Then in April, 1943, she became weak, pale and icteric and had a low grade fever. On examination she appeared chronically ill. There were many round hemorrhages in the ocular fundi. Lymphadenopathy was not present. There was a rough blowing systolic hemic murmur over the entire precordium. The spleen was considerably larger, reaching almost to the level of the umbilicus, while the liver was now palpable 4 fingerbreadths below the right costal margin. The eosinophilia was no longer present. The erythrocytes were of normal size and spherocytes were not present; the acid hemolysis test (Ham) was negative; the cold hemagglutinin titer was 1/10; there were no hemolysins present (added complement) for homologous or heterologous (group O) erythrocytes; the Donath-Landsteiner test was negative and the serum formol gel reaction was negative. Quantitative urobilinogen excretion determinations revealed 571.9 mg./day (average of four days) in the stool and 3.38 mg./day (average of two days) in the urine. A blood culture (fourteen days' incubation under 10 per cent carbon dioxide) showed no growth. The brucellin (Lederle) test failed to reveal dermal hypersensitivity. There were no agglutinins in the serum for *Brucella abortus*

or *melitensis* and the opsonophagocytic test was likewise negative. Roentgenographic study failed to reveal lesions in the skull, bones of the extremities or the lungs.

During the two week period before splenectomy the temperature varied from 98.6 to 100.4 F. The spleen was found to be adherent and there were no accessory spleens. It weighed 1060 gm., was grayish red and moderately firm. There was an infarct at the upper pole and the pulp was not hemorrhagic. The pathologic diagnosis was "granulomatous process resembling the spleen of brucellosis". There were many round granulomas formed of large immature reticulum cells, eosinophils, lymphocytes and polymorphonuclear leukocytes. Several small lymph nodes present at the hilus of the spleen appeared normal. Clinical improvement was steady as the pallor and icterus subsided. Two weeks after splenectomy the urobilinogen excretions were normal, i.e., 95 mg./day in the stool (average of four days) and 0.85 mg./day in the urine (average of two days).

Five months later (November 1913), she was feeling well but had observed the presence of enlarged cervical lymph nodes. These were hard, movable and discrete, measuring up to 4 cm. in greatest dimension. The splenectomy scar was well healed and the liver was 2 fingerbreadths below the costal margin. The cardiac murmur had almost completely disappeared. The platelet count was now elevated to 800,000 and the differential blood count revealed segmented neutrophils, 65 per cent; nonsegmented, 5 per cent; segmented eosinophils, 5 per cent; basophils, 1 per cent; lymphocytes, 12 per cent; monocytes, 12 per cent.

The terminal episode occurred in January 1914, when she developed diarrhea, dyspnea, orthopnea, cough and fever. The anterior and posterior cervical lymph nodes were markedly enlarged, firm, fixed and not tender. There was a trace of albumin in the urine with occasional white blood cells, red blood cells and granular casts. The blood protein now totaled 4.9 gm. with 2.8 gm. albumin and 2.1 gm. globulin. Tests for brucellosis were again negative. Her condition progressively declined with the temperature varying between 99 and 106 F. Sulfamerazine therapy was without effect. Sixteen x-ray treatments, each of 75 r directed over the lower portions of the lungs and the right upper abdominal quadrant, were likewise ineffectual. The symptoms noted on admission continued unabated and the patient succumbed.

The pertinent autopsy findings included some lymphadenopathy with firm to calcific discrete nodes in the cervical, tracheobronchial, paratracheal, mesenteric, periaortic and porta hepatis regions. Lymphatic elements were largely replaced by a collagenous reticulum in which multinucleated cells, eosinophils and foci of necrosis or chronic inflammation were observed. The heart was not enlarged but was flabby. There was a considerable increase in the periadventitial cells. In the lungs there were many dark nodules measuring 5 to 8 mm. in diameter. Microscopically, there was an extensive, highly vascularized loose connective tissue reaction which appeared to start in perivascular locations and involved the septa. Cellular nodules in which numerous mitotic figures could be seen were observed in this granulation tissue. The kidneys likewise contained a number of nodules of histiocytes, multinucleated cells, lymphocytes and occasional polymorphonuclear leukocytes. Areas of severe focal necrosis with polymorphonuclear leukocytes and giant cells were present in the bone marrow. The over-all impression of the histopathologic changes was atypical Hodgkin's disease.

CASE 7

Lymphosarcoma

M. T., a 55 year old woman, died of hemolytic anemia after an illness of six months despite the fact that splenectomy had been performed. She was of English extraction, from a family free of hemolytic disease, anemia, jaundice or splenomegaly and she had not been exposed to known toxic agents.

Six months before coming under our observation she noted the onset of weakness, drowsiness, dyspnea and epigastric fullness. Soon after she was told that she was jaundiced. Four months later she entered another hospital, where a diagnosis of hemolytic anemia was made and roentgen examinations of the chest and gastro-intestinal tract were found to be negative.

On examination at The Mount Sinai Hospital, she was observed to be pale and moderately icteric but showed no evidence of weight loss. The papillae of the tongue were atrophied. There were no enlarged lymph nodes. The heart was enlarged to the left and a hemic systolic murmur was present over the precordium. The spleen was palpable 3 fingerbreadths below the left costal margin; its edge was firm, blunt and not tender. There was slight tenderness in the right upper abdominal quadrant but the liver could not be felt. There were no hemorrhagic phenomena.

TABLE 3
HEMATOLOGIC FINDINGS IN CASE 6

DATE	HB. PER CENT	ERYTH- RO- CYTES MIL- LIONS	LEUKO- CYTES	RETIC- ULO- CYTES PER CENT	HE- MATO- CRIT	HYPOTONIC SALINE FRAGILITY OF ERYTHROCYTES		BONE MARROW PER CENT NORMO- BLASTS AND ERYTH- RO- BLASTS	ICTER- US INDEX	COMMENTS
						Begin- ning	Com- plete			
Jan. 14, 1943	92	5.09	6,000	0.9	43.0	0.44%	0.34%	28.5		Pruritus. Biopsy: focal chronic lymphadenitis.
Apr. 29	38	2.10	2,300	14	21.5			67.6	14	
May 10	52	2.30	3,500	19		0.44%	0.28%			Low grade fever.
May 14		Splenectomy. 500 cc. blood transfusion								Pathologic report: granuloma sug- gestive of bru- cellosis.
May 16		500 cc. blood transfusion								
May 24	71	4.06	10,400	1	36.0	0.44%	0.24%		6	
Nov. 5	85	4.65	9,800	0.5				7.3	10	Afebrile. Biopsy: acute and chron- ic lymphadenitis
Jan. 20, 1944	88	4.47	28,400	0.5						Lymph node bi- opsy: granu- loma, most prob- ably Hodgkin's disease.

Study of the peripheral blood showed the hemoglobin to be 30 per cent; erythrocytes, 1,145,000; leukocytes, 22,350; platelets, 180,000; nonsegmented neutrophils, 14 per cent; segmented neutrophils, 50 per cent; segmented eosinophils, 2 per cent; lymphocytes, 27 per cent; monocytes, 5 per cent; myelocytes, 2 per cent. There were 15 normoblasts per 100 leukocytes and 30 per cent reticulocytes. The hematocrit was 17 per cent and the mean corpuscular volume, 148 cubic microns. There were no spherocytes, the erythrocytes being primarily macrocytic and somewhat hypochromic. Basophilic stippling was evident. The fragility test of the erythrocytes revealed hemolysis beginning in 0.48 per cent sodium chloride and complete hemolysis in 0.24 per cent solution. Other hemolytic tests were negative,

namely, the acid hemolysis test, the heat resistance test, the cold hemagglutinin test (titer 1 to 5) and test of the patient's serum for agglutinins and hemolysins at 37 C. Hemoglobinemia was not present. The sedimentation rate of the red blood cells was 150 mm. in one hour (Westergren); the serum formol gel reaction was negative; the icterus index was 30; the plasma prothrombin index was 100 per cent. Chemical examination revealed: non-protein nitrogen 30 mg., total serum proteins 7.0 gm. with a normal albumin-globulin ratio, negative direct van den Bergh reaction, cephalin-cholesterol flocculation between 1 and 4 plus and alkaline phosphatase 16 King-Armstrong units per 100 cc. The bromsulphalein test (2 mg. per Kg.) showed 15 per cent retention after thirty minutes. Sternal bone marrow obtained by aspiration showed a total nucleated cell count of 235,000 per cu. mm. with 11 megakaryocytes per cu. mm.; neutrophilic myelocytes 9.2 per cent; erythroblasts, 7.2 per cent; normoblasts, 60.8 per cent. Several urine examinations revealed normal findings except for the urobilinogen which was increased to 1/150 (Wallace-Diamond).

Prior to splenectomy, which was performed one week after admission, five blood transfusions were given without reaction. The spleen was markedly enlarged, weighing 1470 gm. The tail of the pancreas was firmly adherent at the hilus and several enlarged lymph nodes were observed. The pulp of the spleen was dark red and fleshy, showing several infarcts, scattered hemorrhages and tiny irregular whitish foci. Microscopic examination revealed lymphosarcoma in the spleen and lymph nodes, in addition to the splenic "arterial" hyperemia characteristic of hemolytic anemias.

The immediate reaction to the operation was relatively favorable despite the deepening of jaundice and the appearance of bilirubinuria. One week after operation the hemoglobin was up to 56 per cent and the reticulocytes had fallen to 12 per cent. Nevertheless, the moderately febrile course which was present persisted and became more marked after the first week. Twelve days after operation it was clear that hemolysis was again active. The hemoglobin was 42 per cent and the erythrocyte count was 1,810,000; there were 46 normoblasts per 100 leukocytes and the reticulocytes had risen to 35 per cent. The excessive urobilinogenuria persisted. Soon thereafter persistent vomiting set in, the temperature rose and the patient died of uncontrolled hemolytic anemia sixteen days after splenectomy.

Prior to the examination of the pathologic specimen there was no indication of lymphosarcoma or of any other disorder except hemolytic anemia and, while the morphology of the red blood cells was most unusual, the decision to perform splenectomy was forced by the relentless progress of the anemia.

CASE 8

Colloid carcinoma

A. J., a 57 year old woman, was studied during two admissions to this hospital both before and after splenectomy. Twenty-three years previously, she had an appendicostomy for chronic ulcerative colitis of unknown etiology and was in fair health until four months before coming under observation. Stool evacuation occurred through the artificial stoma. Jaundice was first noted four months before admission, followed by pallor, fatigue, dizziness and loss of 10 pounds in weight. There was no previous jaundice or anemia that could not be accounted for by her colitis. The family history was negative for anemia, jaundice and splenomegaly and the patient had not been exposed to toxic agents. She was chronically ill. Prolapsed intestine was present at the site of the appendicostomy. The liver was palpable 2 fingerbreadths below the right costal margin and the spleen 2 fingerbreadths below the left costal margin. There was an apical cardiac systolic murmur.

The blood findings are given in Table 4. There was marked spherocytosis of the erythrocytes. The Wassermann and Kahn tests were negative. Blood chemistry showed icterus index, 17; van den Bergh direct and indirect, negative; gastric analysis revealed free hydrochloric acid; urinalyses were negative. There was a negative acid hemolysis test (Ham),

a titer of 1/5 of cold hemagglutinins and an absence of hemagglutinins (37 C.) or hemolysins against homologous or heterologous group O erythrocytes. Examination following aspiration of the sternal bone marrow revealed a total nucleated cell count of 310,000 per cu. mm.; 110 megakaryocytes per cu. mm.; neutrophilic myelocytes, 25 per cent; nonsegmented neutrophils, 17 per cent; erythroblasts, 13 per cent; normoblasts, 35 per cent.

Prior to splenectomy she had a temperature between 100 and 101.5 F. The spleen was enlarged, free of adhesions and weighed 337 gm. The transverse colon was the site of an active inflammatory reaction. The wall was markedly thickened and the serosal surface was red and granular. Microscopically, the spleen showed active hyperemia with marked congestion of the pulp and empty sinusoids, as seen in hemolytic anemia. There were, in addition, multiple foci of hematogenous tuberculosis but tubercle bacilli could not be found. The early postoperative course was smooth. The temperature fell to normal as the jaundice and reticulocytosis receded and the blood count improved. The spherocytosis continued, however. A partial remission of the hemolytic process was evident.

TABLE 4
PERIPHERAL BLOOD STUDIES IN CASE 8

	HEMOGLOBIN PER CENT	ERYTHRO- CYTES MILLIONS PER CU. MM.	LEUCO- CYTES THOUSANDS PER CU. MM.	RETICU- LOCYTES PER CENT	HEMATO- CRIT	FRAGILITY OF ERYTHROCYTES		NORMO- BLASTS PER 100 LEUKO- CYTES
						Beginning Hemolysis	Complete Hemolysis	
Admission	29	1.29	28.0	61	14	0.52%	0.16%	3
2 weeks later	29 (1 T.)	0.97	7.8	40	13	0.52%	0.12%	
3 weeks later	Splenectomy (2 T.)							
4 weeks later	65	3.25	8.0	4		0.52%	0.24%	
18 weeks later	24 (2 T.)	1.02	8.3					51
19 weeks later	47 (3 T.)		9.5			0.60%	0.28%	142
21 weeks later	28 (3 T.)	0.92	15.7	75	15	0.60%	0.20%	143
24 weeks later	27 (1 T.)	1.01	21.0	87	16			370
27 weeks later	29	0.85	24.0	82		0.72%	0.12%	129

T. indicates number of blood transfusions given, each of 500 cc.

The patient remained well for about twelve weeks and then relapsed clinically and hematologically. Examination revealed evident pallor and slight icterus. The liver was still considerably enlarged. Rectal examination was not possible because of stricture. The results of the blood studies are given in Table 4. Of special note is the uncontrolled severe anemia despite multiple blood transfusions given without reactions and the administration of liver extract and parenteral vitamin therapy. There was an intensification of the spherocytosis as is shown by the increase in fragility of the erythrocytes. At the same time a progressively more marked reticulocytosis had occurred. Sternal bone marrow obtained by puncture at this time showed megaloblasts, 3.5 per cent; erythroblasts, 12 per cent and normoblasts, 42 per cent. Carcinoma cells were not present. Further studies were made in an attempt to elucidate the etiology of the relapse. The roentgenograms of the skull and thorax were negative. The basal metabolic rate was plus 47 per cent. The plasma hemoglobin concentration was less than 5 mg. per cent. The temperature varied between 99 and 101 F. Two months after her second admission numerous irregular masses were palpated in the lower abdomen and a hard plaquelike infiltration of the skin was felt adjacent to the appendicostomy. Small hard nodules were present in the scar of the splenectomy. "Punch biopsy" near the stoma revealed colloid carcinoma. At the time of discharge from the hospital the hemoglobin was 25 per cent. Two months later the patient died at home. There was no autopsy.

This is a case of acquired spherocytic hemolytic anemia in a patient with long-standing chronic ulcerative colitis. In addition, hematogenous tuberculosis and colloid carcinoma were present. There is a possibility that the latter arose in the chronically diseased colon. The hemolytic anemia was temporarily benefited by splenectomy, but the spherocytosis was unchanged. With continuation of disease there was a severe recurrence and accentuation of the hemolytic process.

Many authors have described the effect of splenectomy in symptomatic hemolytic anemia. An immediate operative mortality of 20 per cent is reported in these patients. In a case of chronic lymphatic leukemia¹¹ an exacerbation of both the hemolytic anemia and leukemia followed the operation. Temporary improvement in the anemia after splenectomy in cases of Boeck's sarcoid, Hodgkin's disease, lymphatic leukemia and myeloid leukemia is reported.^{1, 4, 10, 13, 23} More beneficial and lasting effects are also described.^{15, 16, 29}

As pointed out previously, the pathologic changes in the spleen in symptomatic hemolytic anemia are variable. This variability does not seem to be a crucial factor in the clinical response to splenectomy. Complete cessation, partial remission, temporary remission and no effect on the hemolytic tendency have been observed in cases with each of the various types of splenic change. The expectation that patients with "arterial" hyperemia of the spleen might show the most dramatic cessation of hemolysis following removal of the organ is not borne out.

The persistence of spherocytosis of the red blood cells despite cure of the anemia after splenectomy in congenital familial hemolytic anemia is well established. The disappearance of spherocytosis of the red blood cells with cure of the disease after splenectomy in idiopathic nonfamilial hemolytic anemia has been observed on frequent occasions. This evidence is used in differentiating these two types of hemolytic anemia. Only passing mention has been given in the literature to the effect of therapeutic measures on spherocytosis in symptomatic hemolytic anemia. Disappearance of this abnormality was observed by Introzzi, Quirno and Pavlovsky¹⁵ and by Singer and Dameshek.²³ In Brill's case¹ the erythrocytes were less fragile (presumably less spherical) after splenectomy. Feldman and Yarvis¹⁰ noted no change in spherocytosis, whereas Crane and Zetlin⁴ stated that as the anemia improved the spherocytosis disappeared; later both reappeared in marked degree. In our Case 5 spherocytosis persisted after splenectomy and radiotherapy despite improvement in the anemia. Eight years after the operation, when the hemolytic anemia was no longer present, spherocytosis had disappeared. In Case 8 spherocytosis remained during the early postoperative period when improvement in the hemolytic anemia was evident. Subsequently, with the inception of a severe relapse, the spherocytosis became more marked. We have also observed spontaneous disappearance of spherocytosis in Case 9.

The question of spontaneous remission of the hemolytic anemia in these cases assumes considerable importance because little attention has been paid to this in the literature. One of our patients with chronic lymphatic leukemia (Case 9) exhibited a striking degree of remission in various elements of his hemolytic

anemia with no therapy other than transfusions of blood. Over a two month period 5000 cc. of blood was administered in preparation for splenectomy. Because this therapy did not significantly alter the level of hemoglobin from the initial value of 26 per cent, the patient was transferred to a hospital for chronic diseases. He exhibited marked spherocytosis, reticulocytosis and increased fragility of his erythrocytes. In this institution, without further therapy except for 1000 cc. of blood transfused over a four month period, there was a remarkable improvement in his clinical and hematologic status. Spherocytosis and increased fragility of the erythrocytes were replaced by macrocytosis and normal fragility; the hemoglobin which could not be elevated over 30 per cent by previous vigorous transfusion therapy rose to 55 per cent and the reticulocytes receded spontaneously from 47 per cent to 8 per cent. Although some excessive hemolysis may have remained at this date, it is probable that most of his anemia was now related directly to the chronic lymphatic leukemia. Unfortunately, it was not possible to follow this patient further.

CASE 9

Chronic Lymphatic Leukemia

A. M., a 71 year old jeweler, had traveled extensively in the tropics and had had several attacks of malaria. He became ill with palpitation, slight ankle edema and exertional dyspnea nine months before admission to this hospital. His symptoms were progressive. Three weeks before admission, he noticed painless swellings in the neck, axillae and inguinal regions. There was no personal or family history of splenomegaly, anemia or jaundice and he had not been exposed to known toxic agents. He appeared pale, chronically ill and icteric. There were many hemorrhages in the right ocular fundus and a generalized lymphadenopathy. The findings in the lungs and heart were normal. The blood pressure was 130/60. The liver was palpable 3 cm. below the right costal margin and the spleen was felt 2 cm. below the umbilicus.

Roentgenologic examination of the chest revealed old fibrotic tuberculosis with shrinkage and calcification of the right upper lobe, but no evidence of recent disease. The mediastinal lymph nodes were not enlarged. Blood studies revealed: hemoglobin, 26 per cent; erythrocyte count, 1,500,000; leukocyte count, 375,000; platelets, 190,000; nonsegmented neutrophils, 4 per cent; segmented neutrophils, 1 per cent; lymphocytes, 94 per cent; lymphoblasts, 1 per cent; reticulocytes, 47 per cent; hematocrit, 13 per cent; erythrocyte sedimentation rate, 155 mm. in one hour. Many spherocytes were observed; the saline erythrocyte fragility test revealed hemolysis starting in 0.72 per cent sodium chloride with complete hemolysis in 0.28 per cent solution. There were no hemolysins or agglutinins demonstrable at 37 C., or at 4 C. for homologous or heterologous compatible erythrocytes. The acid hemolysis (Ham) and heat resistance tests were negative; the plasma hemoglobin was less than 5 mg. per cent. Numerous other blood studies, during the two months of his hospital stay, revealed essentially similar findings. The severe anemia, reticulocytosis, spherocytosis and increased hypotonic saline fragility of the erythrocytes persisted during this period of observation despite ten blood transfusions (5000 cc.), none of which was followed by any reaction.

The jaundice varied somewhat during this period, repeated determinations of the icterus index varying between 12 and 21 with a negative direct van den Bergh test. Blood chemistry was normal. The sternal bone marrow obtained by aspiration was examined on two occasions. In one examination the lymphocytes numbered 71.4 per cent and the normo-erythroblasts 19.4 per cent, while later there were 36.4 per cent lymphocytes and 38.8 per cent normo-erythroblasts. Quantitative determination of the total urobilinogen excretion

over a four day period (Watson) revealed daily excretions of 1.06 mg. in the urine and 252.4 mg. in the stools. Throughout this patient's course he was comfortable in bed and practically afebrile. Splenectomy was not performed because at no time could his hemoglobin be raised over 30 per cent. Radiotherapy was not given.

The patient was discharged to a hospital for chronic diseases. When last examined, four months after discharge, his condition was remarkably good. The only therapy consisted of two blood transfusions (1000 cc.) in the past four months, the last, one month ago. He was ambulatory and free of edema and exertional dyspnea. His color was much improved. The lymphadenopathy, splenomegaly and hepatomegaly were unchanged. The blood findings showed hemoglobin, 55 per cent; erythrocytes, 2,225,000; leukocytes, 117,000; platelets, 240,000; reticulocytes, 8 per cent; nonsegmented neutrophils, 2 per cent; segmented neutrophils, 4 per cent; lymphocytes, 91 per cent; hematocrit, 27.8 per cent. The fragility test of the erythrocytes to hypotonic saline revealed beginning hemolysis in 0.44 per cent sodium chloride and complete hemolysis in 0.18 per cent solution. There were no spherocytes. The red blood cells were definitely macrocytic and well filled with hemoglobin. The icterus index was 8.

During the first period of observation, this patient presented all the criteria for the diagnosis of both chronic lymphatic leukemia and spherocytic hemolytic anemia. After discharge to another hospital and without any therapy, except for an occasional blood transfusion, the spherocytosis, increased fragility of the erythrocytes, marked anemia, moderate icterus and intense reticulocytosis were replaced by macrocytosis, essentially normal fragility with moderate anemia, icterus and reticulocytosis. At the same time, the general condition of the patient improved greatly. The status of the chronic lymphatic leukemia remained unchanged. The cause of this dramatic change in the clinical and hematologic status of the patient has not been ascertained.

In another case of probable chronic lymphatic leukemia a similar sequence took place. This patient (Case 10) had a macrocytic hemolytic anemia. The only therapy consisted of 1000 cc. of blood in two transfusions. One month later, there was evidence of a complete remission of the hemolytic phase, while elements of the underlying chronic lymphatic leukemia became more prominent. One year later there was neither anemia nor evidence of excessive hemolysis and the general clinical condition of the patient was good. The chronic lymphatic leukemia was more evident.

CASE 10

Chronic Lymphatic Leukemia

J. G., a 51 year old man, had been under observation for one year because of a severe acute hemolytic anemia with generalized lymphadenopathy. The cause of the latter was undetermined at the time, but subsequent examinations indicated probable chronic lymphatic leukemia. He was admitted to this hospital with a history of pallor, malaise and anorexia for three weeks and fever for one week. Three months before admission, he had pneumonia which was treated with a sulfonamide. He was employed as an electrician, but had not been in contact with known noxious agents. There was no family or personal history of anemia, splenomegaly or jaundice.

On examination, he appeared pale and slightly icteric but not acutely ill. The lymph nodes in the cervical, axillary and inguinal regions were firm, discrete, movable and enlarged, measuring from 2 to 5 cm. in greatest dimensions. There was a soft systolic murmur

at the pulmonic area. The blood pressure was 98/74. The liver was palpable 2 cm. below the right costal margin but the spleen could not be felt.

Examination of the peripheral blood revealed: hemoglobin, 33 per cent; erythrocytes, 1,600,000; leukocytes, 8100; platelets, 220,000; segmented neutrophils, 40 per cent; nonsegmented neutrophils, 17 per cent; segmented eosinophils, 2 per cent; segmented basophils, 1 per cent; lymphocytes, 26 per cent; monocytes, 12 per cent; neutrophilic myelocytes, 2 per cent; 1 normoblast per 100 leukocytes; 15 per cent reticulocytes; mean corpuscular volume, 120 cubic microns; sedimentation rate of erythrocytes, 18 mm. in eight minutes (Wintrobe). The serum formol gel test was negative. The fragility test of erythrocytes with hypotonic saline revealed beginning hemolysis in 0.48 per cent sodium chloride and complete hemolysis in 0.18 per cent solution. Spherocytes were not present. The acid hemolysis test and tests for hemolysins against homologous or heterologous erythrocytes were negative. The cold hemagglutinin titer was 1/10. Bone marrow obtained by sternal puncture was highly cellular. The total nucleated cell count was 550,000 per cu. mm., and megakaryocytes numbered 110 per cu. mm. There were myelocytes, 9.6 per cent; nonsegmented neutrophils, 6.4 per cent; segmented neutrophils, 1.0 per cent; lymphocytes, 4.0 per cent; hematogones, 3.6 per cent; erythroblasts, 1.6 per cent; normoblasts, 69.0 per cent. Quantitative determination of the urobilinogen excretion revealed 510 mg. per day (average of four days) in the stools and 9.74 mg. per day (average of two days) in the urine. The sheep erythrocyte agglutinin titer was less than 1/5. The Wassermann test was negative. Numerous urinalyses revealed traces of albumin, occasionally, and of red blood cells, rarely. There was a progressive decline in the icterus index from 12 at the time of admission to 3 just before discharge. The direct van den Bergh reaction in the serum was negative. X-ray films of the chest disclosed neither parenchymal disease of the lungs nor mediastinal lymphadenopathy. Biopsies of right axillary and left inguinal lymph nodes revealed chronic or hyperplastic lymphadenitis.

During the first week of his hospital stay, the temperature varied from 100 to 102 F.; thereafter it was normal. During this period, there was a slight increase in the hemoglobin to 38 per cent. He then received two 500 cc. blood transfusions, without reactions, following which there was rapid clinical and hematologic improvement. One month after admission, the hemoglobin was 75 per cent, the erythrocyte count 4,620,000 and the leukocyte count 6000. The lymphocytes were now 66 per cent and the reticulocytes 0.5 per cent. Study of the bone marrow now revealed a considerable increase in the more mature neutrophils to 50 per cent and in the lymphocytes to 20.8 per cent whereas the normoblasts and erythroblasts combined had decreased to 14.4 per cent. The fecal urobilinogen excretion fell to 170 mg. per day and the urinary urobilinogen to 0.72 mg. per day.

One year later, his general condition was excellent. Large retroperitoneal lymph nodes were palpable deep in each iliac fossa. There were no other lymph nodes and the spleen could not be felt. Examination of the blood showed hemoglobin, 91 per cent; erythrocytes, 5,740,000; leukocytes, 10,000; platelets, 260,000; segmented neutrophils, 40 per cent; nonsegmented neutrophils, 2 per cent; segmented eosinophils, 3 per cent; segmented basophils, 1 per cent; lymphocytes, 51 per cent; monocytes, 3 per cent; and reticulocytes, 0.5 per cent. The icterus index was 3.

This patient progressed through a phase of acute hemolytic anemia of unknown causation which subsided spontaneously after two blood transfusions. The lymphocytosis in the peripheral blood and bone marrow and the persistent lymphadenopathy indicated that he was probably affected with chronic lymphatic leukemia or follicular lymphoblastoma which might become manifest in the future.

Other observations regarding spontaneous remission of hemolytic anemia concern a patient with giant follicular lymphoblastoma (Case 3). A partial remission was also noted in a patient with chronic lymphatic leukemia (Case 2).

It may be of importance that all of the patients in whom we have observed spontaneous or transfusion-induced remissions of their hemolytic syndromes had, in addition, chronic lymphatic leukemia (3 cases) or giant follicular lymphoblastoma (1 Case).

The following are the only references to spontaneous remissions recorded in the literature. Lucey¹⁹ described a case of carcinoma of the lung with extensive metastases in which there was evidence of leuko-erythroblastosis in addition to severe hemolytic anemia with increased fragility of the erythrocytes. Without any therapy there was a remarkable hematologic improvement in which the hemoglobin rose from 36 to 70 per cent, the erythrocyte count rose from 1,350,000 to 4,500,000 per cu. mm. and other signs of hemolytic anemia waned. This improvement had no decisive effect on the clinical course for the patient died soon thereafter. Davidson⁵ studied a patient with Hodgkin's disease and hemolytic anemia with increased fragility of the red blood cells and an intense reticulocytosis in whom there was a temporary slow and gradual improvement in the anemia while the patient was receiving liver orally. The hemoglobin rose from 22 to 60 per cent while the red blood cell count rose from 840,000 to 3,470,000 per cu. mm. Simultaneously there was a fall in reticulocytes. Three months later there was a recurrence of the severe anemia and the patient died.

Table 5 summarizes the findings in the 10 cases reported in this paper.

BLOOD TRANSFUSIONS

We have indicated those cases in which blood transfusions may have played a rôle in the induction of a remission of hemolytic anemia. In our patients, a total of 72 blood transfusions was administered by the indirect technic. No special precautions were taken to prevent transfusion reactions. In most instances, citrated blood from the hospital bank was used and cross matching was performed by the slide technic. In many of the patients, Rh typing was performed, but some were observed before the discovery of this blood factor. On three occasions, in two different patients, pyrogenic reactions occurred, but these were not severe enough to discourage further transfusions. In no instance was a hemolytic reaction observed. In many other types of hemolytic anemia we have been impressed with the tolerance to transfused blood. If the correct precautions are taken in blood grouping, including Rh typing and cross matching of the donor's erythrocytes and the recipient's serum, and in the preparation of transfusion equipment and solutions, adverse reactions to blood transfusions are no more likely in patients with hemolytic anemia than they are in other patients requiring multiple transfusions. The only exception to this is the rare case in which atypical agglutinins are present in the recipient's blood serum.

TREATMENT OF SYMPTOMATIC HEMOLYTIC ANEMIA

1. If the hemolytic component is severe and overshadows the underlying disease, and the condition of the patient does not warrant delay, splenectomy without preoperative radiation should be performed. Radiation to other involved areas may be given at a later date. When the anemia is of hyperacute

TABLE 5
SUMMARY OF FINDINGS IN PATIENTS WITH HEMOLYTIC ANEMIA AND OTHER DISEASES

CASE	DIAGNOSIS	LESIONS IN SPLEEN	SPHEROCYTOSIS OF ERYTHROCYTES	MACROCYTOSIS OF ERYTHROCYTES	THERAPY	REMISION OR RESPONSE TO THERAPY
1	Boeck's sarcoid	Not known	Present, previously absent	Absent	None	Previously complete remission
2	Chronic lymphatic leukemia	Not known	Present	Absent	Blood transfusions	Partial remission
3	Giant follicular lymphoblastoma	Not known	Absent	Absent	Radiotherapy, blood transfusions	Previously partial remission; no response to therapy
4	Hodgkin's disease	Not known	Absent	Slight	Radiotherapy, blood transfusions	None
5	Hodgkin's disease	"Arterial" hyperemia	Present, later absent	Absent	Blood transfusions, splenectomy, radiotherapy	Complete remission induced by radiotherapy after splenectomy
6	Hodgkin's disease?	Granuloma	Absent	Absent	Splenectomy, radiotherapy, blood transfusions	Complete remission following splenectomy
7	Lymphosarcoma	Lymphosarcoma, "arterial" hyperemia	Absent	Present	Splenectomy, blood transfusions	Temporary partial remission after splenectomy
8	Colloid carcinoma, healed hematogenous tuberculosis, ulcerative colitis	"Arterial" hyperemia, hematogenous tuberculosis	Present	Absent	Splenectomy, blood transfusions	Temporary partial remission after splenectomy
9	Chronic lymphatic leukemia	Not known	Present, later absent	Absent, later present	Blood transfusions	Partial remission
10	Chronic lymphatic leukemia?	Not known	Absent	Present	Blood transfusions	Complete remission

onset, splenectomy may be impossible, and frequent blood transfusions should be administered.

2. In patients with lymphoblastoma, who are in good general condition, we recommend radiotherapy to the spleen and to any other areas involved in the disease process. If the hemolytic process continues unabated or becomes more severe, splenectomy, followed by postoperative radiation, if necessary, should be carried out. These considerations may be modified by the use of radioactive elements for internal radiation.

3. Patients whose hemolytic anemia continues unchanged after splenectomy should be subjected to thorough study in order to ascertain the presence of neoplastic disease. If found, this should be treated by radiation or extirpation, where feasible.

4. There are no precedents for the successful treatment of symptomatic hemolytic anemia complicated by metastatic carcinomatosis. It would appear that the principles already mentioned should be applied to such cases.

5. Transfusions of properly grouped and matched blood may be given repeatedly to patients with symptomatic hemolytic anemia as supportive or pre-operative medication.

SUMMARY

Hodgkin's disease, chronic lymphatic leukemia, reticulo-endotheliosis, metastatic carcinomatosis, sarcoma of the spleen, myelogenous leukemia, lymphosarcoma, giant follicular lymphoblastoma and Boeck's sarcoid are occasionally complicated by symptomatic hemolytic anemia. The blood picture and bone marrow in such cases may reflect changes caused by both diseases. Spherocytosis and increased fragility of erythrocytes in hypotonic salt solution occur in about 50 per cent of the cases. The pathologic changes in the spleen are variable. In some cases, "arterial" or active hyperemia is present; in others the specific changes of the underlying disease are observed; in certain instances "nonspecific" alterations, such as reticulo-endothelial hyperplasia, erythrophagocytosis and myeloid metaplasia are seen. The reason for this variation is not clear; it is not dependent upon any of the hematologic findings. The unpredictable effect of, and indications for, various forms of therapy are discussed. The occurrence of spontaneous remission is pointed out. The summaries of 10 cases illustrating the clinical and hematologic findings and response to treatment are given.

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THE FUNCTIONS OF THE WHITE BLOOD CELLS*

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Recent advances in our knowledge concerning the rôle of the various mature leukocytes of the blood have been of such promise as to warrant a review of the functions of these cells at the present time. The leukocytes, arising as they do in the tissues of the blood-forming organs, are found in the blood only in passing, and exert many of their more important functions after they have left the blood and entered the tissues. Leukocytes may be found in the blood and in the tissues in different states of activity and, thus, may differ in their chemical and physical status and even in the structural evidences of their various functions. Leukocytes studied only in the blood itself may show marked differences in these structural evidences of their function. For example, we may cite the claim of Aschoff and Kiyono⁵ that the lymphocytes of the blood did not belong to the reticulo-endothelial system because they were not phagocytic for the colloidal dyes under the circumstances of their experiments. However, the brief but pointed experiments of Downey,^{46, 47} which followed soon after, demonstrated that the lymphocytes within the blood itself were capable of phagocytosing the colloidal dyes, if the dyes were made available to these cells by the simple expedient of making a double ligature of a vessel and studying the leukocytes in the interposed segment. Structural similarities in the leukocytes of different species may mask cytochemical disparities in function. For example, the neutrophils of man and the heterophil leukocytes of guinea pigs, rats and rabbits show marked alkaline phosphatase content, while those of the mouse, chicken, and dog lack such enzymatic activity (Wachstein¹⁸²). Cytochemical studies of leukocytes have received marked impetus from the recent contributions of Gomori,⁷⁰⁻⁷² which have done much to facilitate studies of enzymatic activity.

In the study of some of the more fundamental cytologic processes, because of the very nature of the studies, it has not been possible to separate the activities of the different leukocyte types. For instance, Stephens and Hawley¹⁶¹ noted the high content of ascorbic acid (indophenol reducing substance) in white blood cells, and this was confirmed by Butler and Cushman,²² so that there is at the present time the suggestion that the ascorbic acid content in the leukocytes is a better index of physiologically significant deficiency than ascorbic acid levels in the urine or blood plasma. Recently, in attempts to shed further light upon this concept, Wilson and Lubschez¹⁹² suggested that prolonged massive dosage of ascorbic acid leads to a depression of the ascorbic acid content of the leukocytes in children.

The following discussion of the functions of leukocytes is arranged largely according to the different groups of mature leukocytes.

* Read at the Regional Meeting on Hematology of the College of American Pathologists, Indianapolis, Indiana, April 7, 1947. Received for publication, May 17, 1947.

NEUTROPHILIC LEUKOCYTES

The first knowledge of the primary defense functions of the leukocytes was obtained through the observations of Dutrochet,⁵³ Addison¹ and Cohnheim,²⁸ who noted their migration from the vessels into areas of inflammation. Lieberkühn¹⁰⁷ demonstrated the ameboid motility of the leukocytes *per se*. Leber¹⁰² was the first to show that the leukocytes exhibited the property of chemotaxis. The phagocytic powers of these cells were first demonstrated by Metchnikoff.^{121, 123} Opie¹³¹⁻¹³³ found a proteolytic enzyme in these cells in inflammatory exudates that acted in a slightly alkaline or neutral medium. Soon after, Winkler¹²³ demonstrated an enzyme, oxidase, in the leukocytes of blood films. The high oxidative metabolism of leukocytes was observed by Grafe,⁷⁴ but these were cells from leukemic patients. Levene and Meyer¹⁰³ soon reported on the high glycolytic activity of leukocytes. Fiessinger's⁶⁴ monograph on the leukocytic ferments concerned lipase, alkaline lecithinase, amylase and the proteolytic ferments. Sehrt¹⁵⁵ found that the granules of the neutrophils stained with sudan III and Nile blue sulfate and believed that the oxidase reaction of blood cells containing lipoid was proportional to the amount of lipoid they contained. He felt that the lipoidal substances were phosphatides, especially cerebrosides, and probably cholesterol esters as well. Nucleotidase was reported in the leukocytes in the sterile pus obtained after intrapleural injection of turpentine in dogs and cows (Deutsch and Rösler³⁵). Kay,⁹⁵ Umeno¹⁷⁸ and Roche¹⁵⁰ brought forth evidence that an alkaline phosphatase ester-splitting enzyme was to be found in leukocytes. Stern¹⁶⁵ next demonstrated catalase in the polymorphonuclears in rabbit exudates. Soffer and Wintrobe¹⁵³ found that the metabolism of granulocytes resembled that of malignant cells and that their oxygen consumption was greater than that of the lymphocytes, as were their glycolytic powers. Warren¹⁸⁴ found that normal myeloid cells possessed a high ratio of anaerobic glycolysis to respiration, unrelated to cellular damage or the presence of tumor. The practical application of his work was the finding that thiouracil caused a marked depression of the respiration of leukocytes.¹⁸⁵

Menkin^{116, 118} correlated a slightly alkaline pH of the inflammatory exudate with the presence of high percentages of polymorphonuclear leukocytes in the inflammatory exudate. Later, he¹¹⁷ demonstrated that the mechanism of the migration of these cells into an area of inflammation was related to a substance, leukotaxine, produced by the injured tissue. Seabra¹⁵⁷ introduced a numerical expression of the power of oxidase in the neutrophils.

Barnes⁹ was able to demonstrate the following enzymes in the leukocytes of rabbits: cathepsin, nuclease, amylase, lipase, lysozyme (leukin) and adenosinase; those of the cat were similar but were lacking in lipase. Wachstein¹⁸² demonstrated varying amounts of alkaline phosphatase activity in normal human neutrophils and increased activity in such cells in individuals suffering from infections, in purulent exudates and in one patient with nonleukemic myelosis. Wislocki and Dempsey¹⁸⁴ found no differential staining of the specific granules for phosphatase in the leukocytes of monkeys, rodents or man; the staining, when it did occur, was variable and involved both cytoplasm and nucleus.

Carrel²⁵ believed that the neutrophils contained growth-activating substances or trephones. Kruschov⁹⁹ has recently extended Carrel's investigations and has observed an accelerating effect of leukocytic trephones in the growth of tissue cultures of fibroblasts, organ cultures and in experimental wounds; in the latter, at first, granulation production was enhanced and later, epithelialisation, by these leukocytic products.

Boros and Leszler^{17a} claimed a rôle in agglutinin production for these cells, but Ehrlich, Harris and Mertens⁵⁵ could find no agglutinins in the isolated granulocytes of peritoneal exudates.

Further studies on the phagocytic functions of the leukocytes indicate that phagocytosis is proportional to the number of collisions between cell and particles, the number of uningested particles, and the probability of collision;¹²⁷ that it increases with increases in temperature within certain limits;⁶¹ and that hypertonicity tends to inhibit it,⁷⁷ as do certain ions such as I ions,⁷⁸ while it is enhanced by other ions such as Ca ions.⁹³ Menkin¹¹⁶ demonstrated the destructive effect that increases in hydrogen ion concentration have upon the protoplasm of polymorphonuclear leukocytes, explaining the earlier findings of Fenn⁶² and Evans⁵³ that phagocytosis is best at neutrality.

The early work of Wright and Douglas¹⁵⁵ established the presence of thermolabile substances, the opsonins, in normal serum which enhanced phagocytosis, and that of Neufeld and Rimpau^{128a} of relatively thermostable antibodies, the bacteriotropins, in immune serum specifically enhancing phagocytosis. This knowledge had its clinical application in the opsonic index. Hanks⁷⁹ recently determined the quantitative influence of the number of bacteria and leukocytes in phagocytic indices. Czekalowski¹³⁰ spoke of two kinds of opsonins: one was a residual phagocytic factor and was thermostable; the other was inactivated by heat and by storage of plasma, but when present, as in fresh plasma, was five times more active than the former. This second factor, lost by storage of blood, could be regenerated within certain limits by the addition of small amounts of fresh plasma.

Diekey and Forbus³⁶ found *in vitro* that the neutrophilic leukocytes of nonimmune persons quickly phagocytosed *Brucella suis* a response not exhibited by other leukocyte types. Recently, Merling¹¹⁹ demonstrated viral phagocytosis and depicted the virus living intracellularly. He found that his intraleukocytic virus did not die, but survived the death of the leukocyte, remaining as colonies. Welch, Davis, and Price¹⁸⁷ studied concentrations of penicillin which inhibited phagocytosis and found that such concentrations were usually not attained clinically in penicillin administration. Hale⁷⁵ observed inhibition of phagocytosis by coagulase and it also caused agglutination of the leukocytes. Berry, Davis and Spies¹³ found an increased magnitude (119-340 per cent) of phagocytic activity of neutrophils in patients with macrocytic hyperchromic or microcytic hypochromic anemias, and the magnitude was roughly proportional to the severity of the anemia. Glenn⁶⁹ found that phagocytic indices of rabbits were increased following radiation over a small area of the skin. Pokrovskaya and Makarov¹³⁷ studied phagocytosis by neutrophils in human wounds and found that in wounds

which healed satisfactorily the microbes were phagocytosed and destroyed, but that in patients having a low resistance or bacteria of marked virulence, the phagocytosed organisms were at times capable of destroying the protoplasm of the neutrophils.

The views of Sehrt¹⁵⁸ on the chemical nature of the neutrophil granules have been mentioned. The extensive review of this subject by Neumann¹²⁹ led him to believe that they were loci of enzymes or enzyme-like substances. Ralph¹³⁹ believed that the granules of neutrophils contained phospholipids and lipids extractable with acetone. Wislocki and Dempsey¹⁹⁴ found sudanophilic properties in the neutrophil granules of the Rhesus monkey; Baillif and Kimbrough⁷ observed increased affinity for sudan black B in toxic neutrophil granules, whereas the granules of neutrophils in pernicious anemia were but lightly sudanophilic. In general, neutrophilic granules are slate gray and probably do not contain the same substance as the shell of the eosinophilic granules.

Wislocki and Dempsey¹⁹⁴ have been able to demonstrate a punctate type of glycogen in the neutrophils, but not in other blood cells. Tullis¹⁷⁷ recently reported permeability studies of the neutrophils and found that these cells retained their structural integrity most often in isotonic and slightly hypertonic solutions. Cytochemical studies of Watkin's "hair cells"¹⁴⁸ (neutrophils having minute nuclear projections) have recently been made by Discombe³⁷ who found that these nuclear buds which protruded from the neutrophil nucleus of cells from normal and diseased patients might split off into the cytoplasm and that they contained desoxyribosenucleic acid.

McCutcheon¹⁴⁴ has recently made an extensive study of the mechanism of chemotaxis in these cells. Chemotaxis for these cells is excited by polysaccharides, bacteria, malarial protozoa and the by-products of injured tissue.

Lewis¹⁰⁵ studied the mode of neutrophil locomotion by means of motion pictures. The rate of locomotion averaged 19.4 microns per minute. He interpreted locomotion as being due to the forward forcing of a more fluid central portion of the cell by the continuous contraction of a more solid portion of the cytoplasm located at the sides and posterior part of the cell. As the more fluid portion is thus forced forward, its own lateral portions become more solid and thus continually contribute to the formation of a new solid contractile peripheral layer. Forward motion is also maintained by liquefaction of the inner surfaces of the more solid portions of the cytoplasm. De Bruyn^{32, 34} extended these studies and found that all blood cells moving on a flat surface presented the "hand-mirror" shape and all those moving inside the plasma clot presented a "worm-like" motion. However, he found the pseudopodal area of the granular leukocytes more variable in position, the pseudopodia more numerous, and the migration path irregular with frequent and abrupt directional changes, and concluded that these cells were polarized, but to a lesser degree, than the lymphocytes. He presented evidence that constriction rings were indentations caused by external factors. He described large lateral protuberances in heterophil leukocytes which remained immobilized while the rest of the cell advanced, the protuberances falling posteriorly until they were taken up in the tail. He, too,

accounted for locomotion as a process of gelation-contraction-solution and suggested that contraction was due to folding or side-chain locking of a three-dimensional reticulum of polypeptides.

EOSINOPHILIC LEUKOCYTES

The association of these cells with the reaction to foreign protein and allergic disorders has long been remarked. We are just beginning to learn, however, how these cells function under such conditions. Barker⁸ demonstrated iron in the granules of human eosinophilic leukocytes as early as 1894. A year later, Mesnil¹²⁰ reported that the eosinophils of the guinea pig were phagocytic. Petry¹³¹ confirmed Barker's studies on the iron content of the granules and extended the cytochemical studies, finding the granules insoluble in fat solvents, unheated acetic acid and dilute alkalies and soluble in strong acids and alkalies. The granules of the eosinophils upon which he was working were those of the horse; they were not affected by trypsin or autolytic enzymes, nor did they give a positive xanthoproteic reaction. Schlect¹⁵⁵ found that eosinophils reacted to peptones but not to amino acids. Schwarz^{156a} in his extensive review of the literature on the eosinophils and eosinophilia up to 1914, stated that eosinotaxis and eosinophilia are produced by the split protein products of exudate or broken down epithelial cells. The phagocytic functions of eosinophils have been studied by Weinberg and Seguin¹⁸⁸ and more recently by Hertzog.⁶³ Bunting²¹ noted that their locomotion was less active and more indirect than that of the neutrophil, and Sabin¹⁵³ also observed that they appeared to advance slower and for less time than neutrophilic cells. Schrt¹⁵⁸ stained the eosinophil granules with sudan III and Nile blue sulfate.

Ringoen¹⁴⁹ stated that they gave both a positive oxidase and peroxidase reaction. Pokrovskaya and Makarov¹⁵⁷ thought it likely that their presence in a wound was a favorable sign. Quite recently, Ralph¹⁵⁹ demonstrated that the granules of eosinophils contained phospholipids. Discombe³⁷ noted that nuclear buds containing desoxyribosenucleic acid might also split off from the nucleus of eosinophils in normal and diseased patients. Wachstein¹⁸² reported that these cells did not show phosphatase activity. Tullis¹⁷⁷ in his permeability studies found that eosinophils were apparently unaffected by the anisotonia of his experiments and deemed that they were "hardy" cells. Baillif and Kimbrough⁷ observed that in eosinophilia occurring in patients with pneumonia, the eosinophil granules were abnormal, imperfectly formed gray granules when stained with Sudan black B, whereas the normal forms consisted of a Sudanophilic, deeply blackened shell and a clear, unstained, Sudanophobic core. Probably the most significant work in helping us to understand the eosinophil function has been that of Code²⁷ who presented evidence that the eosinophils were an important source of blood histamine.

BASOPHILIC LEUKOCYTES

It must be kept in mind that aside from the apparently identical metachromatic staining reaction of their granules, the tissue mast cells and the basophil leu-

kocytes of the blood and bone marrow have nothing in common (Michels¹²⁴). The finding of the properties of chemotaxis⁶⁰ and high heparin content of the tissue mast cells,⁸⁵ as well as the cytochemical studies of the tissue mast cell granules indicating that they are varyingly sudanophilic, show phosphatase activity,¹²⁴ and are the site of the cytochrome C, cytochrome oxidase system and that they lack nucleic acid, lipase, peroxidase, free iron and glycogen¹³⁰ in their granules or cytoplasm, are not applicable at the present time to the basophilic leukocytes of the blood. Actually little is known concerning the functions of the true basophilic leukocytes. Ringoen¹⁴⁸ noted that in experimental inflammations in guinea pigs hematogenous basophils migrated into the subcutaneous tissues and acted as phagocytes but soon underwent rapid destruction. Hertzog⁵³ was unable to find phagocytosis in 150 human basophils he studied, but remarked concerning the difficulty in differentiating between basophilic granules and bacteria. Sabin¹⁵³ noted that their ameboid motion was slower than that of either the neutrophils or eosinophils. Bunting²¹ stated that the granules were oxidase-positive. Sehrt¹⁵⁸ was able to stain these granules with sudan III while Baillif and Kimbrough⁷ were unable to stain the granules with sudan black B.

LYMPHOCYTES

At the present time, although it is not generally known, there is more exact knowledge concerning the function of the lymphocytes than perhaps any of the other white blood cells. In 1888, Metchnikoff¹²² demonstrated that, in his animals with experimental tuberculosis, the lymphocytes of the blood migrated into the tuberculous areas and gradually hypertrophied to form large mononuclears and they in turn formed the macrophages and epithelioid cells. At the International Medical Congress in Berlin, in 1890, Metchnikoff's views were bitterly opposed by the German group. In answer, Metchnikoff's¹²³ lectures on the comparative pathology of inflammation delivered soon after, at the Pasteur Institute, and published in 1892, demonstrated irrefutably the rôle of the lymphocyte as it hypertrophies to form first the large mononuclears and then in turn the hematogenous macrophages. Metchnikoff's views on the lymphocyte received widespread experimental support in the work of Yersin,¹⁹⁶ Ruffer,¹⁵¹ Gilbert and Girode,^{63a} Arnold,⁴ Borrel,¹⁸ Kanthack and Hardy,⁹⁴ Ranvier,¹⁴⁰ and in the long series of classic experiments of Maximow¹¹⁰ beginning in 1902. Beattie,¹⁰ Ziegler,¹⁹⁷ Schwartz¹⁵⁶ and Helly⁸² also supported the views of Metchnikoff and Maximow. The thesis of the German opposition was that the lymphocytes were incapable of ameboid motion and hence could not migrate from the vessels into the areas of inflammation and form the macrophages.^{50, 55} Although Lewis,^{104, 105} a decade or so later, was to make painstaking studies of lymphocyte locomotion, disproving the basis of the unwarranted attack of the German workers, the damage had been done.

Even so, Buday,²⁰ Zieler,^{195, 199} Renaut,¹⁴⁵ Verebely,¹⁷⁹ Fischer,⁶⁶ Babkina,⁶ Homen,⁸⁶ Fiendt,⁶³ Wallgren,¹⁸³ Downey and Weidenreich⁴⁹ and Dubreuil⁵¹ contributed to our understanding of the lymphocytogenous macrophage in the years leading up to Aschoff. Aschoff⁵ in his great contribution to our knowledge of a

general defense system of cells in the body, inadvertently excluded the lymphocytes from his scheme of a reticulo-endothelial system because in his experiments the lymphocytes were not phagocytic for vital dyes. Even while he was performing his work, Tschaschin^{175, 176} was already demonstrating that the small lymphocytes phagocytosed the vital dye until their cytoplasmic content was indistinguishable from ordinary macrophages. Then Downey,^{36, 47} in the experiments mentioned in the introduction to this paper, proved that the lymphocytes were actually members of the reticulo-endothelial system because of their ready ingestion of the vital dyes. Work on the lymphocytic origin of the macrophages was continued by Policard and Desplas,¹³⁸ Bergel,¹¹ Dominici,³⁹ Latta¹⁶¹ and Dan-chakoff and Seidlin.³¹ It was readily demonstrable in the tissue cultures of Maximow,^{111, 112} Timofejewsky and Benewolenskaja,¹⁷²⁻¹⁷⁴ Bloom¹⁶³ and Berman,¹² in the rabbit ear window of Harper;⁸⁰ and in innumerable other observations;^{23, 24, 57, 84, 91, 98, 100, 125, 126, 135, 159, 160, 166, 167, 168, 170, 181, 186} in the studies of phagocytosis of Hertzog,⁵³ in the work of Kolouch,⁹⁷ Taliaferro and Klüver,¹⁶² Finlayson and Latta,⁶⁵ of Plimpton,¹³⁶ Dougherty,^{40, 41} Rey¹¹⁶ and of Good and Campbell.⁷³ It has been shown that the lymphocyte may resemble the rosette of neutral red vacuoles,^{50, 112} at one time thought to be characteristic of the monocyte. More recently, it has been demonstrated by De Bruyn,²³ by means of motion pictures, that as the lymphocyte hypertrophied toward the macrophage stage, even its mode of locomotion gradually changed from the polarized "hand-mirror" manner to that of continuous depolarization characteristic of the macrophage. So much evidence has been brought to bear on the lymphocytic origin of macrophages that Cowdry,^{28a} in the latest edition of his text, stated that the only evidence now lacking is direct observation of lymphocytes undergoing such transformation. Such direct observations have now been made in laboratories by means of experiments in which individual living lymphocytes of man have been observed to hypertrophy in less than one hour into small macrophages, in warm-stage preparations.¹⁴³ The hypertrophy of the lymphocyte to the hematogenous macrophage is accomplished with the following morphologic changes: increase in cytoplasm; increase in phagocytic ability for bacteria, cellular debris and vital dyes; increase in nuclear size, breaking up of coarse chromatin masses into fine angular pieces, increase in parachromatin; increase in number of cytoplasmic neutral red vacuoles with aggregation into a rosette-like apparatus; and increasing evidences of depolarization-locomotion. It should be kept in mind, however, that reticulum cells, histiocytes, clasmatocytes and monocytes are other sources of macrophages.

Not only are the lymphocytes one of the important sources of macrophages, but they perform a second equally important function in the production of antibodies. The formation of antibodies (agglutinins) by the lymph nodes was established by the work of McMaster and Hudaek¹¹⁵ and Ehrlich and Harris.⁵⁴ Lymphocytic hyperplasia accompanying this antibody formation suggested to the latter authors that the lymphocyte itself was a factor in antibody contribution. Rich, Lewis and Wintrobe¹⁴⁷ observed that the cells which proliferated in the spleen in acute splenic tumor and in lymph nodes draining infected tissue

were lymphoid in character and concluded, "that one function of the lymphocyte is concerned in some way with the body's reaction to foreign protein". The demonstration of the presence of antibodies in the lymphocytes was made by Dougherty, Chase and White⁴² and by Harris, Grimm, Mertens and Ehrich.⁸¹ The rate of release of antibody from the lymphocytes was shown by Dougherty and his group^{43, 44} to be under adrenal cortical control. The rôle of the lymphocytes in protein metabolism was established by White and Dougherty^{152, 150} who found gamma globulin as a lymphocyte constituent and demonstrated that the rate of its release was under pituitary-adrenal-cortical control. The lymphocytes were found to contain a protein which even electrophoretically resembled normal serum gamma globulin and furnished this fraction either by cytoplasmic budding or cellular dissolution or by both mechanisms.⁴⁵ Gaidamovich,⁶⁸ however, was unable to find anti-influenzal antibodies in the lymphocytes of immunized rabbits in his studies of antiviral immunity.

Lymphocyte locomotion has been extensively studied by Lewis and Webster,^{101, 105} who found that lymphocytes were the first cells to migrate from explanted lymph node pieces into the plasma clot, and later took up increasing amounts of neutral red. Lymphocytes contained no lipoidal substance that could be stained with sudan III or Nile blue sulfate according to Sehr.¹⁵⁵ Soffer and Wintrobe¹⁶³ found the metabolism of lymphocytes to be similar to normal adult tissues, while their glycolytic power was about one-half that of the granulocytes and their oxygen consumption not quite equal to that of the latter. Victor and Porter¹⁸⁰ observed aerobic glycolysis in normal and leukemic lymph nodes. Dixon and McCutcheon³⁵ and Clark, Clark and Rex²⁶ found that lymphocytes did not exhibit chemotaxis. Reding¹⁴⁴ found an enzyme in lymphocytes which broke down nuclein. Faerber⁵⁹ did not find cytochrome in lymphocytes. Barnes⁹ demonstrated the following enzymes in the lymphocytes of the rabbit and cat: cathepsin, nuclease, amylase, lipase, lysozyme and adenosinase. Brachet¹⁹ abolished the basophilia of amphibian lymphocytes by treatment with ribonuclease. These cells were oxidase-negative.⁵⁰ Thorell¹⁷¹ studied nucleic acid metabolism in the lymphocytes and found that the intracellular nucleic acid metabolism of the large lymphocytes indicated a high intensity of growth. Jassinowsky's^{89, 90} quantitative studies of the intensive lymphocytic migration through the gastro-intestinal epithelium have been enhanced by Andrew's^{2, 3} recent work in which he postulated that the degeneration and mitotic activity of such lymphocytes were comparable to the defensive reaction of Hellman's reaction centers in lymphatic tissue proper.

Ralph¹⁵⁹ found no phospholipids in lymphocytes and Baillif and Kimbrough⁷ found that the lymphocytes did not stain with sudan black B. Wackstein¹⁸² found that lymphocytes of the peripheral blood contained no alkaline phosphatase, but that the nuclei of some of the lymphocytes in tonsils and lymph nodes, as well as in areas of chronic inflammation, showed enzymatic activity. Wislocki and Dempsey¹³¹ have shown that lymphocytes of the spleen, bone marrow and lymph nodes contained phosphatase enzyme, less marked in light centers especially of the reaction type, and almost entirely lacking in lymphocytes of the

peripheral blood; the nature of the substrate determined whether cytoplasm or nucleus took up more of the stain. These authors also demonstrated ribonucleoprotein in the lymphocytic cytoplasm. Tullis,¹⁷⁷ in his permeability studies, found that lymphocytes retained their morphologic integrity longest in hypotonic solutions. Along with the reticulum cells, lymphocytes served as an important source of plasma cells and monocytes.¹⁷

PLASMA CELLS

The peculiar bodies described in tumors by Russell,¹⁵² in 1890, were shown to be acidophilic inclusions in the cytoplasm of the plasma cells by Dubreuil and Favre.⁵² As the acidophilic inclusions became larger the basophilic protoplasm diminished, the nucleus was pushed to the periphery and, in some instances, the cell disintegrated releasing the Russell bodies into the tissues.^{108, 113} By the elaboration of metachromatic basophil granules, plasma cells may differentiate into plasma mast cells (Krompecher^{38a} and Downey^{15a}). Dubreuil and Favre⁵² also demonstrated small vacuoles in the cytoplasm of plasma cells that could be stained with neutral red. Bloom¹⁶ showed that these vacuoles, stained with neutral red, in the plasma cell might arrange themselves in the form of a rosette, an arrangement once believed to be specific for the monocyte with this stain. Hertzog⁵³ could find no evidence of phagocytic activity in these cells. Kolouch⁹⁶ demonstrated that as the antibody titer of the blood rose there was a concurrent transformation of plasmacytic reticulum cells to plasma cells. Kagan⁹² found that hyperproteinemia in some instances was accompanied by an increased production of plasma cells. Lowenhaupt¹⁰⁹ suggested a functional relationship between the occurrence of plasma cells in conditions of physiologic and abnormal increased globulin production.

Dougherty and White⁴⁵ noted development of plasma cells in the bone marrow and lymph nodes following adrenal cortical stimulation, but felt that if these cells were a source of the serum proteins, the lymphocytes were a more important source of serum proteins. Further, they thought that plasma cells might be merely a reflection of the degenerative changes occurring in the lymphatic tissues. Pokrovskya and Makarov,¹³⁷ in their studies of human wound exudates, associated the appearance of great numbers of plasma cells with a decrease in the acidity of the wound and with patients showing ulceration, necrosis and poor healing in general. They believed that the plasma cells degenerated readily without transforming into fixed cells. Ehrich, Harris and Mertens⁵⁵ believed it possible that plasma cells produced beta globulins or related proteins but found no evidence that they produced gamma globulin. Bjørneboe, Gormsen and Lundquist,¹⁴ however, observed a massive plasma cell infiltration of the adipose tissue of the renal sinus of rabbits immunized to pneumococci, and found that extracts of this tissue rich in plasma cells contained much more antibody protein than extracts from tissues poor in plasma cells.

MONOCYTES

Simpson^{161, 162} noted neutral red granule groups in the cytoplasm of the monocyte and believed that they were characteristic of the monocyte. This property

was shared by the plasma cell¹⁵ and hypertrophying lymphocyte,^{76, 112} leukocytoid lymphocyte and other members of the reticulo-endothelial cell system. The monocytes as such were obviously motile and phagocytic,⁸³ and in tissue cultures or areas of inflammation rapidly transformed into macrophages (histiocytes^{15, 67, 128}). The monocyctogenous macrophages differed somewhat in their detailed morphology from the macrophages derived from reticulum cells and lymphocytes, (Rebuck^{141, 142}) but probably did not differ greatly in function. A specific function in tuberculosis has been claimed for these cells by Sabin^{29, 154} and her group. Sehart¹⁵⁸ found droplets staining with sudan III and Nile blue sulfate in monocytic cytoplasm; Ralph¹³⁹ found that monocyte granules contained phospholipids and lipids extractable with acetone; Wislocki and Dempsey¹⁵⁴ demonstrated that the cytoplasm of monocytes of Rhesus monkeys contained a few droplets stainable with sudan black. Wachstein¹⁸² could find no alkaline phosphatase in human monocytes. As observed in tissue sections, monocytes apparently show chemotaxis, (McCutcheon¹¹⁴) but attempts *in vitro* to demonstrate this property were of no avail until the work of Jacoby⁸⁸ with the monocytes of hen blood suggested that the attracting influences for monocytes are present but extend only for short distances (25 microns). The problem of monocytic function in areas of inflammation is intimately concerned with those of the more numerous and more important hypertrophying lymphocytes (lymphocytogenous macrophages) and histogenous macrophages, although it is quite apparent that monocytes themselves possess proteolytic enzymes.¹⁷

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TECHNICS USED IN THE STUDY OF ASPIRATED STERNAL MARROW*

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Aspiration of sternal marrow for biopsy is now generally accepted as a valuable aid in the diagnosis of diseases affecting the hematopoietic system. The advantages of aspiration over trephination or surgical excision of tissue from the sternum are safety and simplicity. Since the development of the method of sternal aspiration by Arinkin,¹ in 1927, most workers have been content with the preparation of direct smears from small amounts of aspirated fluid material. A notable advance in technic, devised by Schleicher and Sharp,¹⁸ in 1937, and later utilized and popularized in this country by Limarzi¹⁰ and others, was the development of a standardized procedure involving the concentration of the marrow cells by centrifugation, before preparing the smears.

The basic equipment is simple, consisting of an aspirating needle with an adjustable guard, a syringe and a suitable tube in which the specimen can be centrifuged. The author uses the Klima-Rosseger needle† which has been modified slightly by various workers. The skin over the sternum is prepared, and the site of aspiration, which is usually in the midline between the second and third inter-spaces, is infiltrated with anesthetic solution, following which the needle is forced into the medullary cavity of the sternum.

There is ample justification for using the sternum as the site of aspiration. It is readily accessible and several studies,^{11, 21} including my own observations of autopsy material over a period of years, have indicated that the qualitative and quantitative conditions in the sternum are accurate reflections of conditions prevailing in the other main sites of hematopoiesis.

The first drop of material is used for the preparation of several smears. This material from the first drop is superior for the study of cytologic details, as the cells have not been subjected to contact with anti-coagulant. I do not recommend the use of saline, plasma, or anti-coagulant solutions which some investigators⁸ use to dislodge the marrow cells prior to aspiration, because of the artefacts created by such manipulations. An additional 1.5 cubic centimeters of marrow material is then aspirated, and this is immediately transferred to a paraffin-lined vial containing a small amount of dry heparin‡ which prevents coagulation. One cubic centimeter of this heparinized marrow suspension is placed in a Wintrobe hematocrit tube and then centrifuged according to standards which vary some-

* From a paper read at the Regional Meeting on Hematology of the College of American Pathologists, Indianapolis, Indiana, April 7, 1947. Received for publication, April 22, 1947.

† Manufactured by V. Mueller and Company, Chicago, Illinois.

‡ Powdered heparin was obtained from Hynson, Westcott and Dunning, Inc., Baltimore, Md.

what with the size and speed of the centrifuge. At the end of the period of centrifugation, usually about five minutes, the specimen will be separated into three or four layers, namely, fat (when present), plasma, nucleated cells and erythrocytes. The heights of the various layers are recorded.

The quantitative volumetric data obtained by centrifugation are useful for diagnosis and interpretation of marrow lesions. This constitutes one of the advantages of the centrifugation method over the older direct smear method of observing the marrow. For example, Limarzi¹⁰ reported that the height of the nucleated cell layer was from 5 to 8 per cent in normal individuals, from 1.5 to 3 per cent in aplastic anemia, 20 per cent in microcytic hypochromic anemia, 30 per cent in hemolytic anemia, up to 40 per cent in pernicious anemia and up to 75 per cent in chronic myelogenous leukemia. Schleicher¹⁵ has reported a range of from 4 to 6 per cent in healthy adults. The recently studied series of normal adults at basal levels by Berman and Axelrod³ indicates that the range is somewhat greater than previously reported. The fat volumes of normal adults have been reported as falling within the range of from 0.5 to 3.0 per cent by Schleicher.¹⁵ The fat is discarded and most of the plasma is removed. The nucleated cell layer is completely removed and mixed thoroughly with a small amount of plasma in a paraffin-lined watch-glass, and the smears of the concentrate of marrow cells are made.

The smears are uniformly cellular when prepared in this manner. The pathologist is thus afforded the opportunity for observing numerous cells in a single field and, with a little practice, he can become familiar with the principal marrow patterns of diagnostic importance. He should try to acquire knowledge of the general pattern of fields in the smear of the concentrate, as he does when examining a tissue pattern from a section of a surgical specimen. He should not resort to observation of individual isolated cells as they appear on the ordinary smear.

Differential counts are useful for detailed analysis of smears, as there is a tendency to overemphasize the rare or unusual cells, but if the primary purpose of the aspiration is diagnosis only, observance of the general marrow pattern usually will suffice. In problems of clinical investigation, however, in which less obvious changes in the patterns are of importance, differential counts are usually necessary. The counts should be based on a minimum of 1000 cells because of the large number of different cell types and the low frequencies of certain cells, such as reticulum cells. Many students of the marrow have attempted to determine the cellularity of this organ by resorting to hemocytometer counts of the fluid aspirated material. The difficulty in interpretation of such data stems from the factor of dilution of marrow material with sinusoidal blood. The magnitude of this dilution factor is, of course, not under control. A survey of the literature regarding counts in normal individuals reveals a wide variation of the reported cell counts, from 7550 nucleated cells per cu. mm.¹⁹ to 216,000 nucleated cells per cu. mm. of marrow material.⁷ Of course, any attempt to determine accurately the numerical incidence of certain cell types, such as the determination of the megakaryocyte count of sternal marrow, as described by Dameshek and

Miller,⁴ is subject to the same type of error, namely, that due to dilution of the marrow material with sinusoidal blood.

The majority of investigators have ascribed importance to the ratio of myeloid leukocytes to erythroblasts in the sample of marrow. This ratio, which is usually called the myeloid-erythroid ratio, was reported by Limarzi¹⁰ to vary from 1.75:1 to 3.5:1 in normal persons. Others have reported ratios of from approximately 1:1²¹ to 6:1.¹⁹ I regard a ratio of from 2:1 to 3:1 as likely to occur in healthy adults at basal conditions. Some authors use an index proposed by Pontoni,¹³ the leuko-erythrogenetic ratio, which is the ratio between the percentage of immature leukocytes to erythroblasts. This would seem to eliminate error due to the inclusion of mature leukocytes derived from the sinusoidal blood, but it admits an error due to exclusion of band form and polymorphonuclear leukocytes which are normal constituents of the marrow parenchyma. It must be emphasized that such ratios are relative, and that without quantitative data such as are obtained from the volumetric analysis of the centrifuged specimen, it is unwise to consider changes in the ratios as being indicative of actual increase or decrease of leukopoiesis or erythropoiesis.

For certain types of investigation it may be useful to determine the relative incidences of mitoses among the cells of various maturation levels, and also the relative incidences of the different stages of mitoses. Some authors^{5, 6, 9, 12} have made extensive use of such statistics. I have rarely found it necessary to resort to this elaborate procedure, as the information obtained usually is similar to that which can be gained by ordinary means.

Judging by reports in the American literature, the possibility of more complete utilization of the aspirated specimen generally has been ignored. A valuable contribution to technic has been the recent re-emphasis of the value of using imprints and sections from the small particles of solid marrow material which usually are obtained in a successful aspiration.^{2, 15, 17} Some of the European hematologists, notably Rohr,¹⁴ have made excellent use of imprints from particles of marrow material. The reason for using imprints is that cells of primitive type which tend to be present in syncytial masses or compact clusters are not always present in the fluid material, but remain intact as cell masses in the solid particles. Recently, Schleicher¹⁵ has outlined his procedure for preparation of sections of the solid particles, which he calls "gross marrow units". Berman and Axelrod² have described a minor modification which has proved practical in their laboratory, as Schleicher's method has proved difficult for technicians who must handle a large volume of surgical and autopsy material by routine methods.

The value of the routine use of sectioned material is shown by the discovery of focal lesions, such as miliary tuberculosis, Boeck's sarcoid,^{16, 20} lymphoblastoma, metastatic tumors, and also by the demonstration of anatomic structures such as blood vessels and sinusoids, lymphoid nodules, and others, which are rarely seen in smears.

There is at present a widespread opinion that patients with idiopathic thrombocytopenic purpura fall into two classes with regard to the marrow findings and the advisability of performing splenectomy. It is thought that a favorable

effect from splenectomy may be expected if the marrow contains a normal or increased number of megakaryocytes, and that a favorable effect cannot be expected if the marrow is depleted of megakaryocytes. I have encountered two cases, however, in which splenectomy was unnecessarily delayed because the smears did not contain the number of megakaryocytes which has come to be regarded as normal. The sectioned material from one such case is shown in

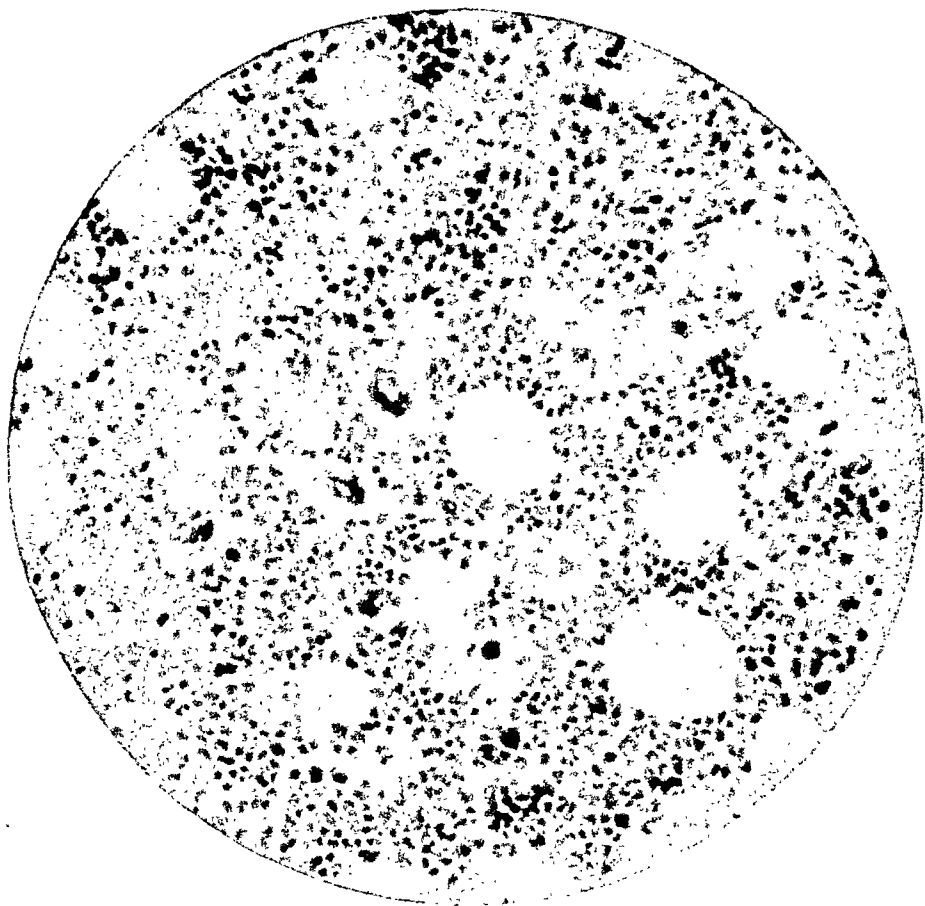


FIG. 1. Section of aggregate of marrow particles from a patient with idiopathic thrombocytopenic purpura showing numerous megakaryocytes.

Figure 1, which is a typical field from the aggregate of marrow particles. In this case, the marrow sections revealed an abundance of megakaryocytes, but the smears contained a reduced number.

I have recently studied a case of monocytic leukemia in which the marrow aspiration was done one week prior to the death of the patient. The marrow sections revealed extensive focal necrosis (Fig. 2) of which there was no clue in the smears. The implications of the last case showing antemortem necrosis are important in view of the great interest in the search for therapeutic drugs for leukemia. If this patient had been treated with an experimental drug, the necrosis which was observed before death would have been present at autopsy,

and there is the possibility that an incorrect assumption would have been made that the change was related to the therapy.

At present, I am carrying out an investigation in which the fat content of the marrow may be a point of importance. Therefore, it has been necessary to make a critical evaluation of the volumetric method for determining the relative fat content of the aspirated specimens.³ The results obtained by the volumetric

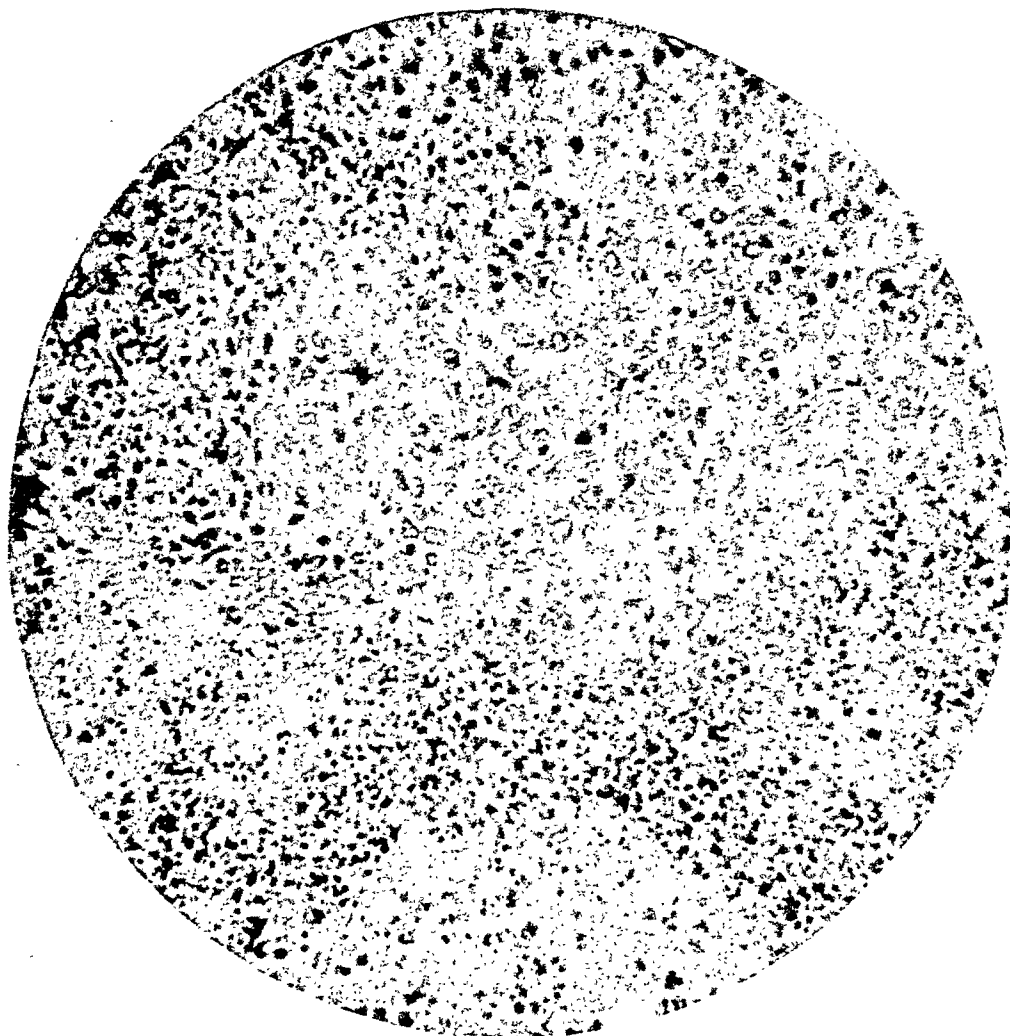


FIG. 2. Section of aggregate of marrow particles from a patient with monocytic leukemia showing antemortem necrosis.

method and those obtained from a study of the sections have been compared. The area occupied by fat spaces is estimated with the aid of a Whipple eyepiece disc.³ There appears to be a rough correlation between the estimate of fat by the volumetric method and the estimate obtained from the sections. There is, however, lack of significant correlation between the estimates by the two methods in instances in which the fat volume is less than or greater than 2.5 per cent.

SUMMARY

The technics of studying material obtained by sternal aspiration, as used in this laboratory, have been discussed briefly, and emphasis has been placed on

the practical value of the utilization of *all* the material obtained. The combined technics, using smears, imprints and sections, makes biopsy of the sternal marrow obtained by aspiration more useful than it would otherwise be if based on a study of smears alone.

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THE HEMOLYTIC ANEMIAS*

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Hemolytic anemias may be classified clinically and etiologically. Clinically, hemolytic anemias are subdivided as hereditary, such as the spherocytic, target cell and sickle cell types, and acquired, which may be either idiopathic or symptomatic, *i.e.*, secondary to coexisting disease. The hemoglobinurias, which are fulminant variants of the hemolytic anemias, are commonly classed as paroxysmal, cold, nocturnal, march and other minor types. Etiologically, hemolytic anemias may be divided into four groups: those due to hemolysins, such as various snake venoms, drugs, chemicals and immune hemolysins; those due to agglutinins as in transfusion reactions and erythroblastosis fetalis, or autoagglutination as in acute hemolytic anemias; those due to disturbances of normal splenic function such as accompany excessive stasis in the organ or abnormal activity associated with a pathologic process; and hereditary groups, such as sickle cell, target cell and spherocytic types.

Several theories have been offered to explain the pathogenesis of hemolytic anemias. It has been assumed that these syndromes are due to defective red blood cell formation, to abnormal function of the reticulo-endothelial system and the spleen, to erythrostasis in the spleen with consequent agglutination and a resulting destruction of erythrocytes, to an increase in lysolecithin or to the abnormal production of hemolysins. It is probable that the hemolytic syndromes are the result, for the most part, of several of these factors which, although acting singly on occasion, usually act synergistically or at least in combination. The most attractive of these theories is the theory of excessive hemolysin production, and my discussion will concern itself primarily with this theory and its clinical and experimental corroboration.

Under normal circumstances, there is a balance between blood formation in the marrow and blood destruction in the reticulo-endothelial system. In the marrow, nucleated red blood cells are produced and these mature to the stage of the reticulocyte, which is extruded into the peripheral stream. Whereas ordinarily the blood reflects the balance between the orderly formation and the parallel breakdown of cells, under abnormal conditions, when blood destruction is excessive either intravascularly or in the reticulo-endothelial system, the balance becomes disturbed and evidences of increased hemolysis occur. Direct evidences are anemia and an increase in blood bilirubin with a secondary increase in fecal urobilin and urinary urobilinogen. There is usually also a spherocytosis

* Read at the Regional Meeting on Hematology of the College of American Pathologists, Indianapolis, Indiana, April 7, 1947. For the sake of brevity, the charts, tables and illustrations used in the talk have been omitted from this paper. Received for publication, April 21, 1947.

of the red blood cells which, therefore, have an increased hypotonic fragility. In instances where the destruction is extremely rapid, hemoglobinemia and hemoglobinuria may also be demonstrated. Indirect evidence of hemolysis is increased marrow activity with consequent peripheral reticulocytosis and polychromatophilia. Nucleated red blood cells appear in the blood in those cases in which blood destruction is very rapid and regeneration is very active. Concomitantly, there is leukocytosis, granulocytosis and an increase in platelets. There is also splenomegaly in most instances.

The two most common types of hemolytic jaundice are the familial spherocytic or congenital type, and the acquired type. The congenital form is transmitted as a Mendelian dominant by either sex, and may become apparent very early in life. As mentioned previously, various theories have been advanced as to pathogenesis. Some believe that the marrow is at fault and is responsible for the formation of the abnormal spherocytes; others believe it to be a reticulo-endothelial disease with phagocytosis of the cells; finally, there is the theory of excessive hemolysin production with secondary spherocytosis. Symptoms may be very slight, and when present may be due to the anemia, the occasional crises which are accompanied by fever, marked weakness and diarrhea, and those symptoms of the gastrointestinal tract, which are usually due to the frequently coexisting cholelithiasis. Most common signs of the disease are pallor, slight icterus, splenomegaly, occasional retardation of growth, "tower skull" and ulcers of the leg. Laboratory findings are variable and depend on the severity of the condition. In mild cases, there is no anemia, while in severe cases, anemia may be marked. The color index is usually about 1.0. The mean corpuscular volume is normal but since the cell thickness is increased, because of the spherocytosis, the mean corpuscular diameter is reduced. For the same reason there is abnormal rouleau formation in wet preparations. Because of the physical changes in the red blood cells, reducing the surface to volume ratio, fragility in hypotonic salt solution becomes increased, and cells are lysed in sodium chloride solution at concentrations varying from 0.5 to 0.85 per cent. Hemolysis also may be complete at higher levels than normal. Reticulocytosis is always present, and its height varies with the severity of the anemia. The marrow is hypercellular with an over-production of red blood cells whose development is normoblastic and shows no "shift". There is bilirubinemia of the indirect type. This is never severe; in uncomplicated cases, the icterus index rarely goes above 30. Liver function tests reveal nothing abnormal.

The causes of acute hemolytic anemia of the acquired type are variable. Although many cases are called "idiopathic", drugs, chemicals, infections and allergies are often incriminated. The anemia has a precipitous onset and is usually accompanied by fever, gastrointestinal symptoms and, not infrequently, by hemoglobinuria. Otherwise, the symptoms are similar to those described for the congenital type. Dyspnea and palpitation, because of the acute onset of the anemia, are more severe, and there is often pain in the region of the spleen. Physical examination is remarkably noncontributory. Except for marked pallor and slight jaundice, there are no constant findings. Frequently there is spleno-

megaly and occasionally hepatomegaly. In acute hemolytic anemias, there are again evidences of acholuric jaundice with hyperbilirubinemia giving the indirect van den Bergh reaction, increased urinary urobilinogen and stool urobilin. The blood and marrow findings are those of congenital hemolytic jaundice in crisis. It is of interest in passing that both the acid hemolysin test of Ham and the Donath-Landsteiner test are negative. On rare occasions, hemolysins and/or agglutinins may be demonstrated in the acute cases.

Of greatest importance in hemolytic anemias is the demonstration of spherocytes. These are red blood cells which have changed from normal biconcave discs into forms which are considerably thicker and, therefore, considerably smaller in diameter than normal erythrocytes, without having changed significantly in volume. Several methods may be used for their demonstration. They can be recognized most readily in the ordinary stained blood smear by virtue of their small size and increased density. They may be identified in the wet preparation by their thickness and inability to form normal rouleau. They may also be demonstrated by cell diameter studies in which the average cell is found to be much below normal, and the first peak in the Price-Jones curve occurs at about 5 microns instead of 7.5. Finally, cells may be shown to be spherocytes by cell thickness studies, based on mean cell volumes and mean cell diameters. The stained smear of congenital hemolytic jaundice will reveal spherocytes and large polychromatic cells, which on vital staining are found to be reticulocytes. The more fulminant of the hemolytic anemias will show, in addition, nucleated red blood cells. The marrow in these instances is markedly hypercellular and is characterized by a preponderance of normoblastic erythrocytes, with the various stages of maturation represented in normal proportions. When a hypotonic fragility test is performed on normal cells, there is no hemolysis at a level of 0.5 per cent salt solution; hemolysis begins approximately at 0.45 per cent and is completed at about 0.25 per cent. When spherocytes are present, however, we find a very much altered situation. Hemolysis now may begin at a level of 0.7 or 0.8 per cent sodium chloride solution and may be complete at a range where, ordinarily, hemolysis would not even have begun.

In studying hemolytic syndromes, and especially questionable ones, the quantitative determination of fecal urobilin and the estimation of the hemolytic index is of invaluable aid. Occasionally, it is also of interest to assay plasma hemoglobin and urine hemoglobin. The acid hemolysin test and the demonstration of isoagglutinins, isohemolysins and autoagglutinins is also helpful in occasional cases.

I should like to review in brief the case histories of four patients with acute hemolytic anemias, in none of whom was a family history of anemia, jaundice, or splenomegaly elicited and in all of whom either autohemolysins, isohemolysins or heterohemolysins were demonstrated.

The first case concerned a middle aged white woman with an acute onset of the classic symptoms. The patient's response to numerous blood transfusions was entirely unsatisfactory. The red blood cell count remained almost as low as it was prior to transfusions and there was very little change in her reticulocyte

counts. Following splenectomy there was dramatic improvement, accompanied by a rising red blood cell count, a falling reticulocyte count and a diminution of the blood bilirubin. During her convalescence, she had two blood transfusions because the erythrocyte count leveled off at a figure below normal. Finally, it was decided to supplement her treatment with liver extract and iron on the theory that the "liver extract principle" might have become exhausted because of the extreme anemia and very rapid blood regeneration. Following this supplementary treatment, and possibly because of it, there was a rise in her red blood cell count which soon reached normal values.

The second patient was also a middle aged white woman who gave a similar response to treatment. In this patient, the time between establishment of the diagnosis and splenectomy was considerably fore-shortened, so she required substantially fewer transfusions before recovery. Much residual hemolytic activity persisted for approximately one month after splenectomy, judging by the patient's reticulocyte curve. Gradually this hemolytic activity diminished, reticulocytes decreased in number, and there was a progressive increase in the red blood cell count, with eventual complete recovery.

The next patient was a white man whose condition was originally incorrectly diagnosed as pernicious anemia. He was intensively treated with liver extract and blood transfusions. Once the correct diagnosis was established, a splenectomy was performed. This operation was followed by dramatic, rapid improvement in both clinical and hematologic states. It is of interest to note that approximately one month after splenectomy he had a secondary reticulocyte rise which probably represented a response to the destruction of the transfused cells which he received prior to splenectomy.

The fourth patient was a boy who was intensively treated with transfusions in the hope of aborting the disease, as described by Lederer in cases of acute hemolytic anemia of childhood. In spite of numerous transfusions, however, the patient eventually had to have a splenectomy. There was a remarkable degree of blood destruction as judged by his inability to maintain a satisfactory red blood cell level in face of the intensive hemotherapy. There was also a marked, constant increase in his urinary urobilinogen before splenectomy and, with the exception of temporary depressions of the reticulocytes following massive transfusions of blood, his reticulocyte level remained between 20 and 40 per cent. Following splenectomy, his post-operative recovery was entirely uneventful and quite complete.

Auto-, iso- and heterohemolysins were demonstrated in these cases and an attempt was made to produce similar changes in animals. Prepared hemolytic rabbit antisera to guinea pig cells were produced and these were injected into guinea pigs in various doses. It was found that when an excessive dose was injected a precipitous drop in the red blood cell count and in the hemoglobin level followed. The drop in hemoglobin, however, was not parallel to the reduction in the number of erythrocytes, because of hemoglobinemia. When somewhat smaller doses of hemolytic sera were administered, the drop in red blood cell count was less precipitous and there was a beginning rise in reticulocytes to

compensate for the blood destruction. Spherocytes appeared about forty-eight hours after the administration of the hemolytic serum and, at the peak of the reticulocyte response, normoblasts also appeared. When still smaller doses of hemolytic serum were given, the above described changes took place more slowly, but the changes were essentially similar. In the course of the drop in red blood cell count, there developed a spherocytosis with a compensatory reticulocytosis. Gradually the dose of hemolytic serum could be reduced to a point where the changes which took place were similar to those seen in chronic human hemolytic anemia. There was constant spherocytosis, moderate anemia and persistent reticulocytosis. If the hemolytic serum administration was discontinued, the animal recovered in a manner exactly similar to that seen in man after splenectomy. The experimental animal may also, in a sense, be immunized to the hemolytic serum by having its blood regenerative activity increased by gradually increasing doses of hemolytic serums. Such treatment produces a functional hyperplasia of the marrow, making it possible for the animal to respond to doses of hemolytic serums which normally would be uniformly fatal. This is similar to what must occur in chronic human cases and illustrates what happens during hemolytic crises in the course of the disease. These hemolytic crises, which ordinarily would be fatal, are tolerated because of the sensitization of the organism by either repeated or continuous previous minor insults.

The reciprocal relationship between red blood cell diameter and red blood cell fragility can be demonstrated readily by producing hemolytic anemias of varying severity. In relatively chronic cases, a great number of spherocytes is produced. At the point where their number reaches its maximum and, therefore, the average cell diameter is smallest, the fragility of the cells is greatest. When administration of the hemolytic serum is discontinued and the spherocytes disappear, the red blood cell fragility returns to normal. This relationship of red blood cell diameter to fragility will vary with the dose of hemolytic serum used. Since the largest doses of hemolytic serums produce most marked spherocytosis, they will also produce the greatest change in red blood cell fragility. Thus, there are analogous processes occurring *in vivo* and *in vitro*, since with each decrease in the average red blood cell diameter there is an accompanying fall in the number of red blood cells.

Of incidental interest in acute hemolytic anemias is the double-humped Price-Jones curve, whose humping is traceable, by differential measurements, to the preponderance of spherocytes on one end of the range and the greatly increased number of reticulocytes on the other end. The configuration of the Price-Jones curve will vary depending on the relative numbers of these two cell types. If cell diameter studies are done on the nonreticulated cells only, in a patient with acute hemolytic anemia, the spherocytes are found to predominate. After splenectomy, however, there is a considerable shift to the right in the Price-Jones curve indicating that the number of spherocytes has diminished and the average cell diameter has increased toward normal. A precise replica of what occurs clinically may be produced experimentally.

From the above described findings, it may be said that hemolytic anemias vary

in their manifestations depending on the amount of hemolysin present. When hemolysin is present in very high concentration, there is a fulminant destruction of red blood cells with hemoglobinemia, hemoglobinuria and a precipitous drop in the number of red blood cells. Somewhat lessened amounts of hemolysin result in a less rapid destruction of cells and a consequent spherocytosis, increased fragility, bilirubinemia and reticulocytosis. When still less hemolysin is present, slower destruction will take place, but the ultimate results, although to a less marked degree, will be the same; thus, there will be spherocytosis and a moderate increase in fragility, but no anemia, since the regeneration can keep pace with the destruction. In these instances, there may be practically no increase in bilirubinemia, although some increase in urobilinogenuria and fecal urobilin will be present.

The evolution of both experimental and clinical hemolytic syndromes may be summarized as follows. During the hemolytic phase clinically, or after the injection of hemolytic serums experimentally, there is a change in the red blood cells which results in the formation of spherocytes. As a by-product there is a marked reduction in the average red blood cell diameter. As a consequence of the spherocytosis the cells become more fragile, disintegrate and anemia results. To compensate for the anemia, large numbers of reticulocytes are released into the circulation and there is a rapid increase in the average red blood cell diameter owing to the presence of these reticulocytes. This represents the regenerative phase, or phase of compensation. With the reticulocytosis there is a concomitant rise in the number of red blood cells. With the increase in reticulocytes and the disappearance of spherocytes, the red blood cell fragility again diminishes and eventually returns to normal as the red blood cell population in turn becomes normal during the phase of recovery.

SENSITIZATION TO THE Hr FACTOR BY BLOOD TRANSFUSIONS*

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The voluminous research, both investigative and statistical, that has been done in relation to the Rh factors has served to acquaint the profession with the importance of these agglutinogens. Less familiar is the reciprocally related antigen designated as Hr by Levine.³ Race and Taylor⁵ also described a serum which agglutinated all Rh-negative cells as well as 80 per cent of the Rh-positive cells, which they named St. It remained for Wiener, Davidsohn and Potter⁸ to demonstrate the similarity in both serums, and to elaborate further on the theory of the occurrence of this Hr factor. As the Hr factor was absent from agglutinogens determined by the genes Rh_1 and rh' , but present in the agglutinogens determined by the genes rh , rh'' , Rh_2 and Rh_0 , the designation Hr' was suggested by Wiener.⁹ That each of the three Rh factors should theoretically have a reciprocally related Hr factor was suggested by Fisher.² Substantiation of at least part of this theory was attained when Mourant⁴ announced the finding of a serum reacting in the manner predicted for anti- Hr'' . The value of these serums in paternity studies was well demonstrated by Wiener.⁷

To date, anti- Hr' testing serum has been obtained from Rh-positive mothers of erythroblastotic children. The infrequency of such sensitization is obvious from the paucity of reports and the scarcity of the testing serums. Statistically among Caucasians in New York City approximately 20 per cent of the population is Hr-negative,⁷ which is a necessary prerequisite for sensitization. The rarity of reported cases of sensitization speaks for the low antigenicity of this factor. The more antigenic Rh factors have been shown by numerous workers to produce sensitization as a result of pregnancy in only one in twenty-five Rh-negative mothers. It recently has been noted that transfusions of Rh-positive blood to Rh-negative recipients produced sensitization in 50 per cent of the cases when repeated transfusions were given. That these same statistical results, but of a lower order of frequency, could be obtained in the Hr series seems plausible. In particular, the transfusion of Rh-negative blood into a homozygous Rh-positive recipient would serve to sensitize the recipient to the greatest degree, as a two gene dose of the Hr antigen is present.

In the operation of a blood bank, the indiscriminate use of Rh-negative blood for Rh-positive recipients has been condemned as wasteful, resulting in unusual shortages of Rh-negative blood when needed. In the light of this report it becomes obvious that in addition to being wasteful, the use of Rh-negative blood for Rh-positive recipients is dangerous. This practice may lead to severe hemolytic reactions in recipients, or to erythroblastosis fetalis in the

* This study was supported by funds provided by Miss Sally Bloom of New York. Received for publication, May 21, 1947.

offspring, and should be condemned for exactly the same reasons that the use of Rh-positive blood for Rh-negative recipients is avoided.

REPORT OF CASE

M. L., a 60 year old white man, was admitted to the surgical service of Dr. Percy Klingenstein with a diagnosis of carcinoma of the cecum. A rather severe secondary anemia (hemoglobin 51 per cent = 8 gm., erythrocytes, 3,200,000) was present and preoperative transfusion was requested by the attending surgeon. The patient's transfusion history was routinely obtained and revealed that four months before admission, he had received an uneventful transfusion of 500 cc. of blood for an unexplained anemia. One week before admission, another transfusion was begun but was terminated after about 400 cc. of blood had been administered because the patient developed a shaking chill, followed by profuse sweating. No observations as to fever, jaundice, hemoglobinemia or hematuria were available.

Routine blood grouping revealed the patient to be group B, Rh-positive. Cross matching with group B, Rh-positive blood from the bank was performed in the usual manner, but was reported as revealing questionable compatibility. Routine slide conglutination

TABLE 1
TITRATION OF Hr ANTIBODY—CASE M.L. (BRh₁Rh₁)

METHOD OF TESTING	TEST CELLS	SERUM DILUTIONS							TITER
		None	1:2	1:4	1:8	1:16	1:32	1:64	
Agglutination	ORh ₁ Rh ₁	—	—	—	—	—	—	—	0
	ORh ₂	++	+	±	—	—	—	—	2
	Orh	++	+	+	±	—	—	—	4
Conglutination	ORh ₁ Rh ₁	—	—	—	—	—	—	—	0
	ORh ₂	++	++	++	+	+	±	—	16
	Orh	++	++	++	++	+	+	±	32

cross matching³ revealed prompt clumping of the donor's cells by the recipient's serum. At this point, it was realized that the patient's serum was acting unusually and more thorough study was undertaken. This revealed the patient to be of group B, Rh₁Rh₁ (Hr negative). A slide sensitivity test³ (conglutination technic) revealed that the patient's serum clumped Rh-negative blood, Rh₂ blood, but failed to clump Rh₁ blood. (This latter subsequently was shown to be Hr-negative.) The results of titration of the antibody in the patient's serum are shown in Table 1.

Testing of several group B, Rh₁ bloods revealed agglutination of fifteen of the twenty specimens tested. The five nonagglutinated specimens were tested with standard Hr' serum and were found to be Hr-negative. Thus, the serum corresponded in specificity to the action of standard Hr' serum. The conglutination titer, however, was higher than the agglutinating titer. This quality made the serum particularly useful as a rapid diagnostic serum for the Hr' factor.

A review of the two previous transfusions received by this patient was attempted. At the time of the initial transfusion, it was found that he had been grouped as B, Rh-positive and had received corresponding blood. The Hr grouping of this blood was unknown. Since only 20 per cent of random bloods are Hr-negative, however, it seems fairly safe to assume that this transfusion was Hr-positive. At the time of the second transfusion, it was discovered that the patient was given O, Rh-negative blood. This resulted in a moderately severe transfusion reaction as described in the patient's history. In the light

of the present findings, a characteristic antibody stimulation or sensitization occurred at the first transfusion, manifested by an antibody-antigen reaction with the Rh-negative Hr-positive cells of the second transfusion. What might have resulted from a third transfusion of Hr-positive cells in this sensitized patient is not difficult to imagine. Instead, a transfusion of 500 cc. of group B, Rh₁Rh₁ (Hr-negative) blood was given with no reaction and a resultant rise of hemoglobin from 8 gm. (51 per cent) to 9 gm. (58 per cent). Subsequently, several transfusions of B, Rh₁Rh₁ (Hr-negative) blood were administered without any untoward reaction.

DISCUSSION

The production of Hr' antibody in an Hr-negative recipient by the transfusion of Hr-positive blood is evidence of the antigenicity of this factor. It thus becomes of increasing importance to determine the Hr reaction of potential recipients of group Rh₁ and rh'. Homozygous (Hr-negative) recipients should receive transfusions of Hr-negative blood only. The dangers of the indiscriminate use of Rh-negative blood in Rh-positive recipients is clearly demonstrated by this case. The Rh-negative blood (being Hr-positive) is as likely antigenic for an Rh-positive, Hr-negative recipient as the reverse would be (Rh-positive blood for an Rh-negative recipient).⁶ The slide agglutination compatibility test has proved its value again when used as a supplement to routine cross matching.

CONCLUSIONS

A case is presented demonstrating a transfusion reaction due to the presence of Hr antibodies as a result of sensitization from a previous Hr-positive transfusion in an Hr-negative recipient. The dangers inherent in the use of Rh-negative blood for any but Rh-negative recipients is demonstrated.

Acknowledgment. The technical assistance of Miss Ruth Brender and Mrs. Hannah Pretshold is appreciated.

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PRELIMINARY REPORT ON A SUBSTANCE WHICH INHIBITS ANTI-Rh SERUM*

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Witebsky and Klendshoj,⁸ Hallauer,⁶ Landsteiner,⁷ Goebel⁵ and others have isolated blood group specific substances of carbohydrate nature from various sources, *i.e.*, gastric juice, red blood cells, saliva and commercial peptone. Belkin and Wiener¹ have demonstrated the property Rh in the stromata of red blood cells and have used the stromata as antigen. Gallagher and Pillischer⁴ have used stromata from group O, Rh-positive cells to immunize guinea pigs to the Rh factor. Calvin and co-workers² have obtained from stromata material which they consider to be a lipoprotein, called clinin, which has Rh specificity. All of these workers with Rh used physical methods for separating the active substance.

It is possible by chemical means to obtain a substance which completely inhibits high-titered standard human anti-Rh serum. This substance is produced as follows: pooled, washed, packed, group O, Rh-positive cells are shaken at 4 C. with an equal volume of distilled water in order to laked the cells. Five volumes of 95 per cent alcohol are added to the laked cells in order to precipitate the protein. The flask containing the material is rotated for thirty minutes and allowed to stand at 4 C. over-night. The red, powdery precipitate is separated from the alcohol by filtration and allowed to dry at 4 C.; 5 volumes of anesthesia ether are added to this powder. The flask is rotated for thirty minutes and kept at 4 C. Extraction continues for eight days, with thirty minutes of rotation daily. At the end of this period, the red powder is separated from the ether by filtration and discarded. The ether filtrate is evaporated with the aid of an electric fan. One six-inch Petri plate is filled with the filtrate and refilled as soon as evaporation will allow. Since the amount of inhibiting substance obtained is small, it is desirable to make all evaporations in the same container.

Complete evaporation of the ether leaves a white to cream colored lipid material. A volume of 800 cc. packed cells yields approximately 300 mg. of the substance. The material is soluble in 95 per cent alcohol, acetone, xylol and chloroform. It seems to be closely associated with the blood cholesterol since it gives positive Liebermann-Burchard and Salkowski's tests. A 1:5 dilution of an anti-Rh standard human serum with a titer of 4 plus in a 1:600 dilution is completely inhibited when this substance is present in a concentration of 0.25 per cent. Some lots have yielded material which would inhibit in a concentration of 0.0125 per cent. This substance does not inhibit high-titered A and B serums.

* Received for publication, April 28, 1947.

The method used for testing inhibition is as follows: 50 mg. of the substance is dissolved in 5 ml. 95 per cent alcohol by shaking gently in a water bath at 56 C. One ml. of this alcoholic solution is mixed rapidly with 1 ml. of 0.85 per cent saline by pouring the mixture back and forth from one vial to another. Equal volumes of this suspension and the Rh serum are combined. The usual practice is to allow the mixture of serum and suspension to incubate at 37 C. for fifteen minutes, but tests show that the inhibition takes place at once. Controls are set up by combining equal volumes of 50 per cent alcohol in saline and the same anti-Rh serum. This mixture is incubated simultaneously with the combination of inhibitor and serum. The inhibited serum and the control serum are tested against a series of group O, Rh-positive cells, as shown below in the usual method used for determining Rh sensitization.

Cells	ORh+	ORh+	ORh+	ORh+	ORh+	ORh+	ORh+	ORh+	ORh+	ORh-
Serum with inhibitor.....	—	—	—	—	—	—	—	—	—	—
Control serum.....	++++	++++	++++	++++	++++	++++	++++	++++	++++	—

The cells used are 2 per cent suspensions, each from a different donor. The serum, before combining with inhibitor or with 50 per cent alcohol in the control, is diluted 1:5. The inhibited serum and the control serum are tested by adding two drops of the serum for each test to two drops of cell suspension in saline. The tests are incubated for thirty minutes at 37 C.

In order to be sure that the inhibition was not merely a physical adsorption of antibody on lipid particles, the suspension of alcoholic inhibitor in saline was centrifuged. The supernatant fluid and the particles resuspended in saline were each used in equal volumes with the anti-Rh serum to see if either alone would inhibit. The supernatant fluid inhibited the serum; the resuspended particles did not. Also the inhibition tests as outlined above were set up using cholesterol (Eimer and Amend, C.P.) in place of inhibitor in the same concentration. No inhibition took place.

Twenty-four guinea pigs were inoculated intraperitoneally and two rabbits were inoculated intravenously with a 10 per cent suspension of this lipid material. Twenty-four pigs were inoculated with 10 per cent suspensions of human group O, Rh-positive red blood cells as controls. Inoculations were made intraperitoneally and the methods previously described elsewhere,³ of injecting on alternate days for a total of six inoculations, were followed for both red blood cells and lipid suspension. Each of the control animals produced agglutinins for group O, Rh-positive cells; the lowest titer was 1:160. None of the animals inoculated with inhibitor substance produced antibodies to human red blood cells. However, when equal volumes of 10 per cent lipid suspension in 50 per cent alcohol and 10 per cent egg albumen (Eimer and Amend, soluble scales) in saline were injected intraperitoneally in the same manner, five out of the twenty-four guinea pigs inoculated developed agglutinins demonstrable in 1:10 dilution for group O, Rh-positive cells. In each of the 72 animals, inoculations of 2 ml. of the appropriate material were made on alternate days

until a total of six injections had been given. Bleedings were made from the heart one week after the final inoculation. Each of these pigs was tested before the series of inoculations was begun to make sure that the normal blood contained no agglutinins for group O, Rh-positive cells. None of the normal pigs showed agglutinins.

As a third check on the specific nature of this lipid inhibitor, tests were carried out to determine whether a suspension of this substance plus human standard anti-Rh serum would fix complement. Extensive experimentation was necessary to determine the antigen dilution range, but finally it proved possible to demonstrate complement fixation with the Rh inhibitor as antigen. In the lot under investigation, 0.5 ml. Rh inhibitor at a 1:2000 dilution with human anti-Rh serum, 0.05 ml. in 0.5 ml. dose, will fix two full units of complement to produce a 4 plus reaction. The complement-fixation tests were set up exactly as in a quantitative Kolmer procedure for the serologic test of syphilis, using carefully titrated lyophilized complement and high-titered amboceptor. Serum controls were negative and, as an additional control, normal human serum was set up in place of the anti-Rh serum in an identical quantitative procedure and tested simultaneously. This control was invariably negative.

Calvin and Behrendt² found their elinin fraction to be thermolabile, but mentioned an ether-soluble material obtainable only occasionally which was thermostable. The activity of our ether-soluble fraction is resistant to heating at 56 C. It is probable that the presence of protein renders the elinin susceptible to heat. When the protein is absent, the inhibitor substance resists inactivation.

It would seem logical to assume that the ether-soluble inhibitor of Rh antibody is hapten. Apparently, in itself, it is incapable of stimulating the production of antibody, but in company with a protein "carrier", Rh antibody is produced. The possibility of making use of Rh hapten in the desensitization of Rh sensitized mothers or in the prevention of sensitization is alluring to many investigators. Work of this type is under way now and will be reported at a later date.

In the process of obtaining inhibitor from red blood cells, it is desirable to save the initial alcoholic filtrate. Some of the inhibitor may be obtained from this by concentration under vacuum.

SUMMARY

A fraction has been separated from group O, Rh-positive cells which is non-antigenic in experimental animals, but which is antigenic when injected simultaneously with a protein carrier. This substance, which is lipid in nature, specifically inhibits the agglutinins present in anti-Rh serum. Also it will fix complement in conjunction with human anti-Rh serum. It resists inactivation by heat and is probably Rh hapten in impure form.

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NESSLERIZATION TECHNIC AND ITS EFFECT ON SERUM PROTEIN VALUES*

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Serum protein determinations in our laboratory by the photometric modification of the Campbell-Hanna method¹ could not be satisfactorily and consistently checked. Duplicate determinations of total serum protein by this method by several workers in the laboratory showed a wide range of values. Our total protein values were invariably high as checked against those values obtained by other reliable methods.^{2, 3}

The Campbell-Hanna method¹ for total serum protein is carried out as follows: Make a 1:50 dilution of serum with 0.85 per cent sodium chloride. Place 1 cc. of the dilution in a digestion tube, 200 x 75 mm., graduated at 21 and 30 cc. Add one glass bead and 0.55 cc. of 1:1 sulphuric acid and digest in a hood as for a nonprotein nitrogen analysis, using four drops of superoxol to complete the digestion. Cook, add distilled water to the 21 cc. mark and place in a water bath at 25 C. Add 9 cc. of Nessler's reagent, mix by inversion, and after five minutes read in a photometer standardized with distilled water at 100 per cent transmittance.

The principal sources of error with the above-mentioned technic are: (1) contamination of the acid digestion mixture with nitrogen compounds; (2) contamination of the superoxol with nitrogen-containing compounds; (3) contamination of samples during digestion due to the presence of nitrogenous gases in the hood; and (4) errors introduced by variations in nesslerizing technic.

To investigate the first three sources of error, three series of blanks using distilled water instead of a nitrogen standard or a serum dilution were set up. These blanks, treated as indicated in Table 1, were read on the photometer which had previously been standardized with distilled water at 100 per cent transmittance. The results are given in Table 1.

While the results shown in Table 1 indicate that the addition of the acid digestion mixture introduces some nitrogen into the sample (approximately 0.005 mg.), the magnitude of the error from this source is negligible. Similarly the data show conclusively that nitrogen contamination from the superoxol or from accumulated gases in the hood is not a significant factor.

Variations in nesslerization technic and their effect on the total protein values were then studied on a pooled sample of serum. Two series of twenty determinations each were run on this sample with all factors of technic controlled and standardized with the exception of the nesslerization process. In series A, the Nessler's reagent was added slowly down the side of the digestion tube and, in series B, the Nessler's reagent was added rapidly and directly into

* Received for publication, May 8, 1947.

TABLE 1

DATA ON THE SOURCES OF ERROR IN THE CAMPBELL-HANNA METHOD FOR TOTAL SERUM PROTEINS

SAMPLES	PHOTOMETER READINGS			
	Series			Average Values
	1st	2nd	3rd	
1. Distilled H ₂ O + heat (in hood) + Nessler's reagent.....	99.4	99.5	99.5	99.5
2. Distilled H ₂ O + heat (outside hood) + Nessler's reagent.....	99.0	99.2	99.5	99.2
3. Distilled H ₂ O + H ₂ SO ₄ + heat (in hood) + Nessler's reagent.....	98.0	98.0	98.2	98.1
4. Distilled H ₂ O + H ₂ SO ₄ + heat (outside hood) + Nessler's reagent.....	98.4	99.0	99.0	98.8
5. Distilled H ₂ O + H ₂ SO ₄ + heat (in hood) + 4 drops superoxol + Nessler's reagent.....	98.0	98.2	98.0	98.1
6. Distilled H ₂ O + H ₂ SO ₄ + heat (in hood) + 8 drops superoxol + Nessler's reagent.....	98.0	98.2	98.0	98.1
7. Distilled H ₂ O + H ₂ SO ₄ + Nessler's reagent (outside hood).....	98.2	98.5	98.2	98.3
8. Distilled H ₂ O + Nessler's reagent (outside hood).....	99.0	99.0	99.3	99.1

TABLE 2

SERIES A: NESSLER'S REAGENT ADDED SLOWLY DOWN THE SIDE OF THE DIGESTION TUBE

SAMPLE	PHOTOMETER READING	MG. N PER CC. OF 1:50 DILUTION	TOTAL PROTEIN IN PER CENT
1	59.0	0.280	8.55
2	60.0	0.271	8.27
3	60.0	0.271	8.27
4	60.0	0.271	8.27
5	58.0	0.290	8.86
6	60.5	0.268	8.18
7	60.5	0.268	8.18
8	58.5	0.285	8.71
9	60.0	0.271	8.27
10	60.0	0.271	8.27
11	60.0	0.271	8.27
12	59.5	0.275	8.39
13	60.5	0.268	8.18
14	61.0	0.263	8.01
15	57.0	0.299	9.14
16	58.5	0.285	8.71
17	59.0	0.280	8.55
18	59.5	0.275	8.39
19	61.0	0.263	8.01
20	59.0	0.280	8.55
Average.....	59.6	0.275	8.57

the center of the solution, with immediate mixing by inversion in both instances. Tables 2 and 3 represent the data from series A and B, respectively.

The pooled sample of serum was analyzed for total protein according to the Looney and Walsh turbidimetric method.³ The value was found to be 6.98 per cent total protein.

TABLE 3

SERIES B: NESSLER'S REAGENT ADDED RAPIDLY AND DIRECTLY INTO CENTER OF THE SOLUTION IN THE DIGESTION TUBE

SAMPLE	PHOTELOMETER READING	MG. N PER CC. OF 1:50 DILUTION	TOTAL PROTEIN IN PER CENT
1	64.0	0.237	7.21
2	63.5	0.240	7.30
3	64.0	0.237	7.21
4	65.0	0.229	6.96
5	64.8	0.231	7.02
6	64.8	0.231	7.02
7	64.0	0.237	7.21
8	65.0	0.229	6.96
9	65.0	0.229	6.96
10	64.0	0.237	7.21
11	64.2	0.235	7.14
12	64.0	0.237	7.21
13	63.5	0.240	7.30
14	64.0	0.237	7.21
15	65.0	0.229	6.96
16	63.0	0.246	7.36
17	64.5	0.232	7.05
18	63.0	0.246	7.36
19	64.5	0.232	7.05
20	65.0	0.229	6.96
Average.....	64.2	0.235	7.14

DISCUSSION

Slow nesslerization down the side of the digestion tube causes a significant elevation of the serum protein level. A value of 8.57 per cent total protein was obtained by this technic, as compared with 7.14 per cent by the rapid addition technic and 6.98 per cent by the Looney and Walsh method.

Early articles by Folin and Denis⁴ for the direct nesslerization technic in nitrogen determinations merely state that the Nessler's solution should be added to the digestion mixture and the resulting solution mixed quickly. Wong⁵ states that the Nessler's solution should be allowed to fall directly into the solution in the digestion tube and then mixed. In the more recent modifications of the direct nesslerization method the directions include only the rapid addition of Nessler's solution to the digestion mixture and immediate inversion to assure complete mixing of the solutions.

CONCLUSIONS

Variations in nesslerizing technic using the Campbell-Hanna method produce marked alterations in the serum protein values.

To obtain satisfactory results in the use of Nessler's reagent, not only should the reagent be added rapidly, but it should be added directly into the center of the solution and immediately mixed by inversion.

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OCCURRENCE OF WALTHARD CELL RESTS OR BRENNER-LIKE EPITHELIUM IN THE SEROSA OF THE EPIDIDYMISS*

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Since it has been recognized that the Brenner tumors are benign growths without endocrine influence, their most interesting aspect lies in the question of their histogenesis.⁵ Meyer¹ believes that the Brenner tumor arises from the so-called Walthard cell rests. Danforth² came to the conclusion that Brenner tumors and Walthard cell rests bear a strong resemblance to one another, especially in nuclear detail; the basic nuclear type of each showing a peculiar groove. Arey¹ after pointing to the longitudinal grooving of the nuclei of the Brenner tumors and of the Walthard cell rests, suggested the serosal epithelium as the common source of both formations. According to Schiller,^{7, 8} the Brenner tumor may develop from the ovarian surface epithelium, especially if it has formed Walthard cell rests, or from remnants of the uropoietic system. Novak⁶ considers the possibility that Brenner tumors may arise from areas of metaplasia of the peritoneum and germinal epithelium. The accidental finding in the epididymis of small nests of epithelial cells which were continuous with the mesothelium of the tunica vaginalis propria and showed a strong resemblance to the Brenner epithelium and the Walthard cell rests may corroborate this conception.

REPORT OF CASE

A Negro male, 38 years old, was admitted to the hospital with a left-sided hydrocele. A diagnosis of filariasis was made. During operation it was decided to perform semicastration.

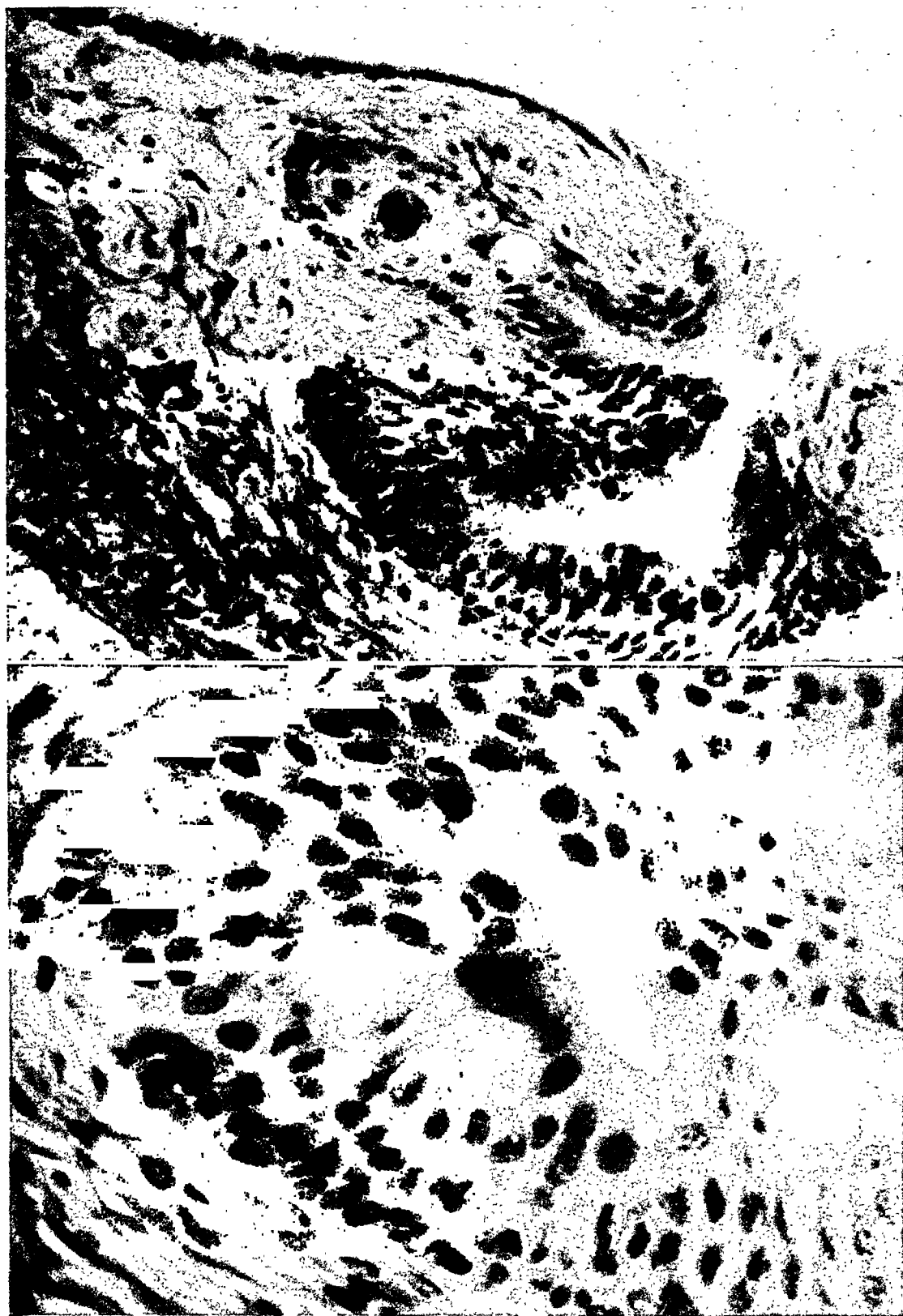
Microscopic examination of the tissue removed showed the interstitial connective tissue of the epididymis to be edematous and infiltrated with small numbers of lymphocytes, histiocytes and numerous eosinophilic leukocytes. In some places, granulomatous endo and perilymphangitis, typical for filariasis, was observed; there was also a large lymph vessel, still recognizable by its muscular coat, which contained a granular necrotic mass, walled off by palisaded epithelioid cells, lymphocytes and eosinophilic leukocytes. In the neighborhood of the lymph vessels the eosinophilic leukocytes and lymphocytes were more numerous. The epithelium of the ductuli efferentes and of the ductus epididymidis was normal; in the latter a few mitoses were found.

Directly below the serosal covering of the epididymis, sometimes in direct continuity with the mesothelial cells or interrupting the mesothelium and projecting a little into the serous cavity formed by the tunica vaginalis propria, there were a few nests and groups of epithelial cells of a peculiar type. The more peripherally located cells were small; they

* Received for publication, May 24, 1947.

Fig. 1. Cell nest, continuous with serosa. One small cavity contains an ovum-like body. x 300.

Fig. 2. Cavity in cell nest lined by cuboidal epithelium. Several nuclei show distinct grooving. x 600.



contained oval nuclei with one or two small nucleoli. Many nuclei showed a longitudinal grooving. In a large cell nest, a cleft, lined by low prismatic epithelium, was seen. There were also small, rounded cavities lined by more flattened epithelium. In several of these cavities rounded eosinophilic bodies, sometimes with a dark-staining inner zone, were found. Others contained a granular precipitate which stained dark red with mucicarmine. The largest cell nest was surrounded by a thin capsule of dense connective tissue.

DISCUSSION

As follows from the description and the photomicrographs, the cell nests showed a strong resemblance to the Walthard cell nests and the epithelium of Brenner tumors. When compared with cell groups of typical Brenner tumors observed in this laboratory,³ no essential differences could be found. Apart from the grooved nuclei and the mucicarminophil substance, even the ovum-like bodies, first described by Brenner, were present. The localization of the cell nests and their continuity with the mesothelium, although not constituting absolute proof, still make a mesothelial origin of the cell nests in this case very probable and, therefore, offer some evidence for the mesothelial origin of the Walthard cell rests and of the Brenner tumors. Findings as described here should certainly be more frequent when a larger amount of carefully fixed material is examined.

SUMMARY

In the epididymis of a 38 year old male, cell nests were found which closely resembled Walthard cell rests and Brenner epithelium. The significance of this finding is briefly discussed.

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CLINICOPATHOLOGIC CONFERENCE*

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A 34 year old white man had had no serious illnesses until September 1945, when he was pensioned from the Army for "aneurysm of the subclavian artery". In February 1946, he developed increasing lassitude, weakness and backache. He received "injections", but gradually developed an unsteady gait and difficulty in walking. In April, he received three transfusions of whole blood. About that time, his abdomen became increasingly distended and he had severe dyspnea when placed on his left side. Diuretics did not relieve the distention. From February to May, his weight decreased from 155 to 135 pounds. He had no other complaints.

Examination revealed a distressed, pale, poorly nourished, dyspneic man. The cervical and supraclavicular lymph nodes were enlarged, especially on the left side. He had a mass in the left supraclavicular region. Pulsations adjacent to the mass were judged to be transmitted from a normal-sized subclavian artery. A hard, pea-sized nodule was felt anterior to the trachea, which was displaced to the left. Small subcutaneous nodules were palpable in the left lateral chest wall, the epigastrium, the lower left quadrant and in the left thigh. The right thorax bulged. Tactile and vocal fremitus were diminished and expansion was limited on the right side. Percussion yielded a flat note on the right, posteriorly, at the level of the fourth thoracic vertebra. The apex beat was felt in the fifth intercostal space, to the left of the midclavicular line. The heart was displaced to the left. The blood pressure was 140/90, bilaterally. The liver edge was palpable 5 fingerbreadths below the right costal margin and was firm and smooth. The abdominal veins were prominent and no fluid wave was elicited.

Course. Between May 8, the date of admission, and May 12, 3200 cc. of turbid fluid was withdrawn from the right pleural cavity. The fluid clotted spontaneously and contained 2.5 gm. of protein per 100 cc. Following thoracentesis, he breathed more comfortably. On May 10, he had some edema of the ankles. On May 14, he coughed up sputum tinged with blood, and on this date, lapsed into coma and expired.

Laboratory findings. Erythrocyte counts, 4,240,000 and 3,900,000 per cu. mm.; hb. 12.5 gm. and 11 gm.; leukocyte counts, 8100 and 4420; differential counts, 63 segmented neutrophils, 21 stab cells, 1 juvenile, 10 lymphocytes, 5 monocytes and 71 segmented neutrophils, 9 stab cells, 2 eosinophils, 15 lymphocytes, 3 monocytes; total protein 4.1 gm. per 100 cc. with a ratio of albumin to globulin of 1.56:1; blood nonprotein nitrogen 36 mg. and urea nitrogen

* Published with permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or the conclusions drawn by the author. Received for publication, February 27, 1947.

18 mg. per 100 cc.; urinalysis was negative. X-ray films of the chest, on May 8, showed obliteration of the shadow of the right lung by fluid. A flat plate of the abdomen revealed free fluid in the peritoneal cavity.

CLINICAL DISCUSSION

Dr. J. Murray Kinsman, Louisville, Senior Medical Consultant. "I have some questions before I start the discussion. First, did he have any fever?"

"He had no fever until the last day, when his temperature reached a peak of 100 degrees."

"Was a Kahn test done?" "Yes. It was negative."

"Was the chest fluid examined bacteriologically?" "No organisms were seen in direct smears. The fluid was not cultured."

"Was the sputum examined for acid-fast bacilli and for fungi?" "The sputum was negative for both, on several occasions."

"Were any agglutination tests done?" "No."

"Were any skin tests done?" "No."

Dr. Kinsman. "Gentlemen, the diagnosis concerns itself with two possibilities, infection and neoplastic disease.

"First to be considered is tuberculosis. The x-ray film of the chest is negative for tuberculosis. He did have fluid in the right pleural cavity, but I believe tuberculosis can be ruled out from the information at hand, and so can syphilis. The other infectious granulomas, except possibly tularemia, are not likely to produce a picture like this. Tularemia does cause lymphadenopathy. I would rule out tularemia, however, since he did not have fever. Actinomycosis, blastomycosis and coccidioidomycosis may involve the skin. When they do, they produce ulcers or sinus tracts. He had no evidence of pulmonary involvement such as is common in fatal fungous infections, so I think we can eliminate them. On the basis of the information available, I would rule out pyogenic infection.

"His illness started with enlargement of the lymph nodes, detected while he was in the service and apparently misinterpreted as aneurysm of the subclavian artery. At least, that diagnosis was not concurred in at this hospital. He also had involvement of the pleural cavity. Fluid removed from the chest contained 2.5 gm. of protein per 100 cc. This value is at the borderline between a transudate and an exudate. Later, an unsteady gait and difficulty in walking indicated that the central nervous system was affected. Therefore, the brain or spinal cord must have been involved by metastases of a malignant tumor. The thing that puzzles me is the presence of subcutaneous nodules. These nodules must have a bearing on the case and, since it is my impression that they are more common in sarcomas than in carcinomas, I am more inclined to favor a diagnosis of a sarcoma. Sarcomas often spread widely and may produce lymphadenopathy with compression and obstruction of blood vessels. Apparently, the retroperitoneal and mediastinal lymph nodes were involved extensively, and presumably the periportal nodes were also. He certainly had involvement of the mediastinal nodes with right pleural effusion.

"Hodgkin's disease can produce this picture, but I'm not sure that it causes

nodules beneath the skin. It usually causes fever at some stage and it produces anemia. The spleen is likely to be enlarged, which was apparently not the case here. It is difficult to differentiate between Hodgkin's disease and lymphosarcoma. I think this man had a malignant neoplasm. I am not sure where it started but the lymph nodes seem to have been involved from the beginning. His age and the distribution of the lesions favor a diagnosis of a sarcoma. To sum it up, I think he had a sarcoma, either Hodgkin's disease or lymphosarcoma."

Resident. "Would the size of the liver influence you in making a diagnosis?"

Dr. Kinsman. "Hardly. Enlargement of the liver may be due to carcinoma or to sarcoma, including leukemia, lymphosarcoma and Hodgkin's disease."

Resident. "What about subleukemic leukemia?"

Dr. Kinsman. "His illness developed rapidly and, if he had had leukemia, I should expect some of the white cells to be primitive."

PATHOLOGIC REPORT AND DISCUSSION

Pathologic diagnosis. Hodgkin's disease, "transitional cell" type, involving the spleen, liver, pancreas, kidneys, adrenals, mesentery, pleura, pericardium and the cervical, mediastinal and abdominal lymph nodes; bilateral hydrothorax; ascites.

Dr. Gordon. "At the outset I confess that we deliberately withheld from Dr. Kinsman the fact that smears of the fluid removed from the chest contained clumps of atypical cells which were interpreted as evidence of Hodgkin's disease. Autopsy revealed a considerable amount of free fluid in the pleural spaces and the peritoneal cavity. The liver weighed 3500 gm. and the spleen weighed 800 gm. Both were studded with nodular infiltrations, microscopically resembling the pleomorphic cells seen in Hodgkin's disease. Similar infiltrations were found in the other tissues listed above.

"Hodgkin's disease, regardless of whether it is considered infectious or neoplastic, is a disease of the reticulo-endothelial tissues and accordingly belongs in the disease group of the 'reticulo-endothelioses'. Apparently, it is not generally appreciated that when Aschoff first coined the term 'reticulo-endothelium', he had in mind only the functional proclivities of widely distributed groups of phagocytic cells. The more recent histologic investigations of Maximow, Metchnikoff and others justify our present concept of a 'reticulo-endothelial system'. This, in turn, makes it possible to classify the diseases of the reticulo-endothelial tissues as truly systemic hyperplasia, according to the following scheme modified from Hadfield, and Garrod."¹

A. The metabolic reticulo-endothelioses (abnormal lipid storage)

1. Etiology unknown

(a) Cerebroside histiocytosis (Gaucher's disease)

(b) Cholesterol-phosphatide histiocytosis (Niemann-Pick disease)

(c) Cholesterol and/or cholesterol ester histiocytoses (essential xanthomatoses)

1. Skeletal. Hand-Schüller-Christian disease

- 2. Cutaneous. Xanthelasma
- 3. Visceral
- 2. Xanthomatosis secondary to (a) chronic jaundice or (b) diabetes mellitus
- B. Hemophagocytolytic reticulo-endothelioses
 - (a) Erythrophagocytolysis with jaundice
 - (b) Leukophagocytolysis with leukopenia
 - (c) Thrombophagocytolysis with thrombopenia
- C. The infectious reticulo-endothelioses (sarcoidosis, certain protozoal infections, leprosy, syphilis, tuberculosis, typhoid fever, and others.)
- D. The neoplastic reticulo-endothelioses
 - 1. Follicular lymphoreticulosis of Brill
 - 2. Medullary reticuloses
 - (a) Undifferentiated
 - (b) Hemic
 - 1. Leukemias
 - 2. Myelomas
 - 3. Lymphosarcoma
 - 4. Leukosarcoma
 - (c) Fibro-myeloid (Hodgkin's disease)
 - 3. Reticular. Reticulo-sarcoma

REFERENCE

- 1. HADFIELD, G., AND GARROD, L. P.: Recent Advances in Pathology. Ed. 4. London: J. A. Churchill Ltd., 1942, pp. 40 and 44.

EDITORIAL

MEDICAL PRACTICE AND COMMERCIAL LABORATORIES OPERATED BY TECHNICIANS

In modern medical practice, the diagnosis is made after complete physical examination, review of the patient's history and after carrying out such diagnostic procedures as roentgenologic examinations, clinical laboratory tests and biopsy. It is, of course, essential that these tests be performed properly and interpreted accurately. In larger centers, radiologists and pathologists, who are duly qualified medical specialists, normally perform this function. In smaller communities, circumstances often require that general practitioners conduct or supervise the performance of technical methods and interpret the findings of roentgen films and clinical laboratory tests.

The profound importance of accurate diagnosis is quite evident in instances of malignant disease. The diagnosis, on biopsy, of a lump in the breast or of a painful lesion in the bone as malignant, will usually indicate the need for amputation of the part in the hope of cure. Who is it that renders the diagnosis and often gives the advice upon which the surgical operation is undertaken? The pathologist. Similarly, in clinical laboratory work, it is the pathologist who is called on to point out the need for performing certain tests, to supervise their proper performance, to interpret the findings and to serve as consultant regarding the further management of the patient.

In the early days of clinical laboratory and radiologic medicine, it was not unusual for technicians to set themselves up as practitioners in these fields. Owing to the shortage of trained medical specialists, these laymen were accepted and supported by the profession at large. In the meantime, departments of radiology and clinical pathology were becoming established in medical schools, and young physicians were being encouraged by the faculties to enter these fields.

Today, if we are to foster the continued development of such competent specialists, we must discourage any further development of laboratories operated by technicians and seek to control those which have become established. In some parts of the country, such laboratories have now become synonymous with convenient diagnoses and even with rebates. It is to the credit of the medical profession that these practices are exceptional. The signs are clear as to what should be done. We should see that all medical laboratories are owned and conducted by physicians trained in clinical pathology. We should not lend aid to laymen who are attempting to invade the practice of medicine, but rather, we should seek to limit the practice of clinical pathology to qualified medical men.

When the existing technician-operated laboratories pass from their present hands, it is to be hoped that the practice of medicine by technicians will become as obsolete as the practice of surgery by barber surgeons. It has not been so many years since all surgery was performed by technicians or barbers and since such practice was condoned by the medical profession. With the growth of medical knowledge, and especially after the development of antisepsis and

anesthesia, the practice of surgery by barbers or technicians was stopped. It now seems appropriate to adopt measures which will expedite constructive evolution in the field of clinical pathology so that the commercial clinical laboratory owned and operated by the technician will likewise pass from the scene.

San Francisco

L. HENRY GARLAND*

THE CLINICAL PATHOLOGIST

The recent past history of medicine is one of brilliant achievement in the fields of scientific and technologic advancement, much of which stems directly from the efforts of a relatively small group of workers whose activities have centered in the experimental and clinical laboratories of the country. These advances have almost revolutionized the practice of medicine and have led to the indoctrination of the profession with the "scientific" method of the study of disease with a resultant marked improvement in the over-all general medical care of the nation. It is in this small group of workers that the clinical pathologists are counted.

Clinical pathology is one of the youngest medical specialties. The early disciples of this specialty were concerned with investigation of the scientific aspects of disease without immediate regard to practical applications, and therefore were attracted to medical schools and research centers, where the teaching and practice of clinical pathology became centralized. Thus, the recent progress of medicine is inextricably related to the development of "clinical" pathology in the large sense, and it can be safely said of the future, "As pathology goes, so goes medicine".

One of the present major indications of the direction in which the specialty is moving is seen in the tendency towards the development of strong decentralized units for medical care. It would seem that the period in which the university with its research center and attached teaching hospital provided the main radiating influence for good medical care in the community is to be succeeded by an era of development of more numerous and widely scattered decentralized units whose function will be to supply the same quality of medical care for a larger proportion of the population. This tendency is already noted from the development of privately operated group clinics, from the recently established experimental diagnostic clinics and from the projected diagnostic clinics under Federal administration. The common denominator of all these attempts is the provision of better scientific care for a greater proportion of the people, and the keystone of this effort is the application of the methods of the clinical laboratory towards the correct diagnosis and treatment of disease.

The active development of the field of clinical pathology represents an important extension of medical care. The clinical pathologist has at his disposal laboratory methods of exactitude with which to determine objectively the

* Dr. Garland is a practising radiologist and is honorary secretary of the California Medical Association.

variations that occur in disease. He receives for diagnosis, as in the biopsy, material obtained by special instruments from areas of the body previously considered inaccessible. He also is able to make important contributions, not only in the diagnosis, but in the therapy of disease. This service is well exemplified in the field of antibiotic therapy, in preservation of water balance of patients in preoperative and postoperative care, in protein nutrition, and in the treatment of shock as controlled by laboratory methods. He further contributes to the educational program of his colleagues by collaborating with the clinical departments in periodic formal and frequent informal clinicopathologic conferences for the purpose of discussing the end results of disease and assaying the accuracy of diagnostic and therapeutic measures.

These various phases of laboratory medicine thus infiltrate the entire medical front and establish the function of the clinical pathologist as one of increasing importance in the general medical scheme. It is vital that this contribution be equally acceptable to the professional, clinical and administrative hospital personnel so that it may receive due emphasis. Furthermore, the individual pathologist making such a contribution should have his efforts placed in the proper perspective, and should command recognition with respect to both the professional and economic aspects of his contribution. The general profession and the hospital administrators of the country should realize the potential effectiveness of this group of medical specialists. Likewise, it is only proper to expect that the members of this group be rewarded in fair proportion to their usefulness and responsibility, as well as to the magnitude of their contribution in the care of the sick.

Meadowbrook Hospital, N. Y.

THEODORE J. CURPHEY

BOOK REVIEWS

Chemotherapeutic and Other Studies of Typhus. Medical Research Council Special Report, Series No. 255. By M. VAN DEN ENDE, C. H. STUART-HARRIS, F. FULTON AND J. S. F. NIVEN, WITH C. H. ANDREWES, A. M. BEGG, W. J. ELFORD, M. H. GLEESON WHITE, W. L. HAWLEY, K. C. MILLS, F. HAMILTON AND C. C. THOMAS. 246 pp., 9 plates, 104 tables. \$3.65. London: His Majesty's Stationery Office, 1946.

This is a report replete with tables and charts describing the greater part of the work on typhus carried on between the years 1941-1946 by the National Institute of Medical Research of London and the Royal Army Medical College Emergency Vaccine Laboratory. For reasons of security, publication was withheld until the end of the war.

The British workers concentrated on chemotherapy and the cultivation of rickettsiae in the mouse lung rather than in the egg. They devised a quantitative test based on the number of visible lesions produced in the lungs, using the previously known fact that pulmonary lesions may be produced in mice by intranasal instillation of various rickettsiae.

Altogether 238 drugs or chemicals including 13 compounds of the sulfonamide group, were tested by the "mouse lung lesion count" method. Two of the drugs, p-sulfonamidobenzamide hydrochloride (V 147) and p-sulfonamidobenzamidoxine hydrochloride (V 186), offered promise experimentally, but when tested clinically in patients were found to be without therapeutic value.

Although the large amount of work reported yields nothing of positive value in the treatment or control of typhus, the volume gives an unabridged account of the symptomatology, clinical course and pathology of epidemic typhus. In the appendix, 13 case histories are given in detail, each well illustrated by temperature, pulse and respiration charts, and tables of laboratory data. Macroscopic and microscopic postmortem results are given for 6 fatal cases.

The chapters dealing with tests for neutralizing antibodies against rickettsiae, comparison of typhus vaccines in the laboratory and the antigenic structure of the typhus rickettsiae will be of more interest to research workers in rickettsial diseases than to clinical pathologists. The clinical pathologist, however, will find in the pages devoted to serology, blood counts and blood chemistry, valuable practical information on the diagnosis of typhus. The bedside slide agglutination test (*Proteus* OX19 suspension with methylene blue dried on a slide) was mentioned favorably and, when strongly positive, indicates a titer of 1:200 or greater.

Baltimore, Maryland

C. H. BINFORD

Physical Medicine in General Practice. Edited by ARTHUR L. WATKINS, M.D., Associate in Medicine, Harvard Medical School; Chief of Physical Medicine, Massachusetts General Hospital, Boston. 341 pp., 15 illus., 3 charts. \$5.00. Philadelphia: J. B. Lippincott Company, 1947.

This timely compilation is reprinted in attractive book form from *Clinics*, April, 1946. Fourteen authoritative contributors, eminent as teachers and researchers in the rapidly expanding field of physical medicine, have performed a yeoman service in bringing together in concise and readable manner the essential facts regarding modern physical medicine.

As Krusen states in the opening chapter, "Physical medicine is at once the newest and the oldest field of medical practice." Physical medicine evolved from milleniums of mixed science and charlatanry during World War I. Between the two World Wars the field of so-called "physical therapy" rapidly gained stature and recognition as a long-neglected field of medical specialization, thanks to the efforts of such indomitable pioneers as Krusen, Coulter, Kovacs and Bierman. The accomplishments of physical therapists in the Medical Corps of the Army and Navy in rehabilitating wounded and sick soldiers, marines and sailors during World War II, and the vision of Bernard Baruch in establishing the Baruch

Committee on Physical Medicine have provided the impetus which has brought the field of physical medicine to its present deserved position of eminence among medical specialties.

The instinctive, empiric belief in the curative merits of heat, for instance, was shared by the ancient Mayans, Egyptians, Romans and Greeks. The chapters by Kendell on Fever Therapy, by Bennett on Poliomyelitis, by Kuhns and Knapp on Orthopedic Disabilities, and by Kovacs on Medical Conditions, among others, provide the scientific bases for such empiricism.

This book will be welcomed by general practitioners, for whom it was primarily intended, but the physiatrist, orthopedist, internist, neurologist, pathologist, psychiatrist, dermatologist, industrial physician and the Army or Navy Medical Officer can ill afford not to have immediate access to it as a desk reference book.

Dayton, Ohio

WALTER M. SIMPSON

La Réticulose Histiomonocytaire. By P. CAZAL, Chef de Clinique de Montpellier. 196 pp., 32 figs., paper. 350 francs. Paris: Masson et Cie, 1946.

The author introduced the term "histiomonocytic reticulosis" in his previous monograph dealing with diseases of the reticulo-endothelial system published in 1942. He defines it as a "disease characterized by metaplastic and systemic proliferation of reticulum cells and histiocytes which may remain localized or may pass into the blood under the form of more or less typical monocytes". The present monograph is divided into four parts. The first deals with the historical aspects and includes the contributions of foreign workers with some American contributions. The second part describes various localizations of the disease. The changes in the blood picture are described as one of the possible localizations of histiomonocytic reticulosis and they are quite variable. In about 35 per cent of cases leukopenia was present. About two-thirds of cases showed a moderate monocytosis (10 to 30 per cent). The third part of the book consists of a general discussion of clinical signs, pathologic anatomy (including a detailed description of various cells) etiology, pathogenesis and briefly, treatment.

Cazal distinguishes the following main clinical forms: (1) acute reticulosis of infants (Abt-Letterer-Siwe's disease); (2) acute, nonleukemic reticulosis of older children and adults; (3) subacute hepatosplenic form, with or without glandular involvement; (4) subacute or chronic glandular form (pseudo-Hodgkin's type); (5) primary cutaneous form, chronic; and (6) acute monocytic leukemia. The last chapter deals with a nosologic discussion. Of the three types of monocytic leukemia, the author rightly includes only the Schilling type as belonging to the scope of histiomonocytic reticulosis rejecting the so-called monocytic leukemia, type Naegeli, and type Marchal. The relation of histiomonocytic reticulosis to the leukemias, erythroblastosis, agranulocytosis, granulomatoses (Hodgkin's disease, mycosis fungoides, eosinophilic granuloma), Still's disease, lipid reticulo-endothelioses and neoplastic diseases of the reticulo-endothelial system is discussed in some detail. Histiomonocytic reticulosis constitutes a definite nosologic entity. A valuable feature of this monograph is the fifty page bibliography comprising abstracts with case summaries of 272 publications from the world's literature.

The general appearance of the book may not particularly appeal to the American reader and many of the black and white illustrations are of rather mediocre quality. In spite of these and a few other minor criticisms, this monograph constitutes a valuable addition to the literature on diseases of the reticulo-endothelial system.

Terre Haute, Indiana

LEON L. BLUM

Allergy in Theory and Practice. By ROBERT A. COOKE, M.D., Sc.D., F.A.C.P., Attending Physician and Director of the Department of Allergy, Roosevelt Hospital, New York City. 572 pp., 43 figs. \$8.00. Philadelphia: W. B. Saunders Company, 1947.

Dr. Cooke has written what is probably the most complete book on allergy available today. It assumes nothing and explains everything in terms that are fundamental. Here is not an expounding of complicated theory but rather a taking-stock of known basic

knowledge in allergy. The contributors are well chosen and include those who are themselves expert in the various fields of allergy. They discuss their special spheres simply and always with a view to practicality. It is particularly satisfying to the practicing physician to find in this book not only the theoretical bases of allergic reactions, but specific instruction on how to treat and when not to treat. The section on technic is outstanding in its thoroughness.

In general, the book is divided into sections on fundamentals: allergy of the bronchi, allergy of the upper respiratory tract, allergy of the skin, allergy of the nervous system, allergy of the cardiovascular system, allergy in relation to other specialties, allergens in relation to diseases of allergy and, finally, the section on technics.

Of special interest to the pathologist is the chapter on pathologic-anatomic aspects of allergy by Dr. Paul Klemperer. Dr. Klemperer makes it clear that allergy from the standpoint of the pathologist cannot be diagnosed from the anatomic-histologic evidence alone. His discussions of anaphylaxis, bronchial asthma, serum sickness, periarteritis nodosa, rheumatic fever and glomerulonephritis are written with understandable restraint, but leave little doubt as to the allergic bases of the pathologic lesions produced. His restraint in discussing the allergic backgrounds of these controversial entities is even better appreciated when he states: "It seems to me that pathologic anatomy must be extremely cautious in the evaluation of tissue lesions in reference to etiology. The pathologist must be exact in his investigations and first establish all the facts which the microscope can reveal. He must try to correlate these facts with the facts established by the clinician. . . . Allergy is a dynamic process which provokes certain tissue changes, which in themselves are not characteristic. To try to prove existence of a dynamic process from purely morphologic observations must lead to sterile speculation."

Detroit

MARK DALE

Essentials of Endocrinology. Ed. 2. By ARTHUR GROLLMANN, Ph.D., M.D., F.A.C.P., Professor of Medicine and Chairman of the Department of Experimental Medicine, The Southwestern Medical College, Attending Physician and Consultant in Endocrinology, The Parkland Hospital, Dallas, Texas. 644 pp., 132 illus. \$10.00. Philadelphia: J. B. Lippincott Company, 1947.

In the second edition of Dr. Grollman's book on endocrinology, he states: "As in the earlier edition, an attempt has been made to develop the subject on the basis of its scientific background rather than on purely clinical concepts." This approach leaves something to be desired by students and physicians who are looking for clear-cut clinical pictures of the endocrine diseases. The author, however, does provide the reader with the physiologic background of glandular diseases and thus enables him to understand the pathologic physiology.

References follow each chapter. About two pages are devoted to the antithyroidal agents. Tables that will be useful to both student and practitioner are to be found on the front and back linings of the book. These are entitled: "Some Commercially Available Endocrine Products and Their Standardization". The term "thyroid extract" instead of "thyroid substance" is erroneously used throughout the book. "Thyroxine" is misspelled on the front lining. There are 132 illustrations in the second edition, as compared to 72 in the first, and most of them are concerned with pathologic changes.

Endocrinology is a difficult subject to cover in any book and like all works on this subject the personal opinion of the writer colors the viewpoint and, naturally, exceptions may be taken. The book can be recommended to furnish the essentials in the study of the endocrines, as the title signifies.

Detroit

ROBERT C. MOEHLIG

OBITUARIES

GEORGE THOMAS CALDWELL

George Thomas Caldwell, of Dallas, Texas, died January 20, 1947. He was born in Kennard, Ohio, in 1882, was graduated from the Rush Medical College in 1919, and was formerly associated with the department of pathology at Rush and at Baylor Medical College. Dr. Caldwell was consulting pathologist at Bradford Memorial Hospital for Babies, Parkland Hospital and Texas Scottish Rite Hospital for Crippled Children, Surgeon (R) in the United States Public Health Service and professor and chairman of the department of pathology at Southwestern Medical College. He was certified in clinical pathology and in pathologic anatomy by the American Board of Pathology and was a member of the American Society of Clinical Pathologists from 1927, of the Texas Society of Pathologists and of the American Association of Pathologists and Bacteriologists.

MAURICE JOHN COURET

Maurice John Couret died in New Orleans on February 4, 1947, where he was director of laboratories at the Hotel Dieu. He was born in New Orleans in 1874. Obtaining his medical degree from Tulane University in 1896, Dr. Couret interned at Charity Hospital in New Orleans, did postgraduate work in pathology at Harvard University and studied at the University of Berlin and the Pasteur Institute of Paris. He was formerly pathologist at the Illinois Central Hospital, director of laboratories at Charity Hospital and assistant professor of pathology at Tulane. During the first World War, he served in Italy as pathologist of Base Hospital 102, with the rank of Major. A bronze plaque was recently unveiled in the Hotel Dieu laboratory to commemorate his work in that institution. His original work included studies on *E. histolytica*, hog cholera, *Balantidium coli* and rabies. Dr. Couret joined the American Society of Clinical Pathologists in 1938, was a past president of the Louisiana Association of Pathologists and a diplomate of the American Board of Pathology.

CORNELIUS ALBERTUS HOSPERS

Cornelius Albertus Hospers, of Chicago, died February 20, 1947, of coronary thrombosis at the age of 42. Dr. Hospers received his medical degree from the School of Medicine of the Division of the Biological Sciences of the University of Chicago in 1932. He interned at the University of Chicago Clinics and was on the staffs of the Holy Cross and South Chicago Community Hospitals. During World War II, Dr. Hospers served as a lieutenant colonel in the medical corps. He was certified by the American Board of Pathology, was a member of the American Association of Pathologists and Bacteriologists and, since 1938, a member of the American Society of Clinical Pathologists.

NEWS AND NOTICES

THE COLLEGE OF AMERICAN PATHOLOGISTS

The first regional scientific meeting of the College of American Pathologists was held in Indianapolis, April 7, 1947, at the Indiana Medical Center in conjunction with the State Pathologic Society. The auditorium was filled to capacity by more than 125 pathologists from six states and the faculty, house staff and undergraduate students. Nine papers which were devoted to problems of hematology were read. The speakers were: Lawrence Berman, M.D., Detroit; Lewis R. Limarzi, M.D., Chicago; Steven O. Schwartz, M.D., Chicago; W. W. Zuelzer, M.D., Detroit; E. A. Sharp, M.D., Detroit; John W. Rebuck, M.D., Detroit; B. K. Wiseman, M.D., Columbus, Ohio; I. Davidsohn, M.D., Chicago; and Edith L. Potter, M.D., Chicago.

Dr. Frank W. Hartman presided at the meeting and at the dinner which followed. After dinner informal talks about the work of the College and plans for its development were made by Dr. Hartman, Dr. A. S. Giordano, Dr. Lall G. Montgomery, Dr. S. E. Gould and Dr. M. G. Westmoreland, executive secretary of the College.

Officers of the College are: Dr. Frank W. Hartman, Henry Ford Hospital, Detroit, president; Dr. Granville A. Bennett, University of Illinois, Chicago, vice-president; and Dr. Tracy B. Mallory, Massachusetts General Hospital, Boston, secretary-treasurer.

"CREDO" OF THE MICHIGAN PATHOLOGICAL SOCIETY

The following "credo" was adopted by the Michigan Pathological Society at its meeting in Saginaw on May 31, 1947. This is to serve as a guide in planning future activities in connection with the practice of pathology in Michigan.

1. We believe that all practice of clinical pathology and anatomical pathology should be conducted by and under the direction of a recognized pathologist. A recognized pathologist shall be one who is certified by the American Board of Pathology or one who has the basic training required for certification.

2. We believe that the state laws and regulations should be revised to recognize that the practice of clinical pathology and anatomical pathology is a branch of the practice of medicine.

3. We believe that the operation of a clinical or pathological laboratory should be considered as the "practice of medicine" and as such should be subjected to the provisions of the basic law known as the Medical Practice Act.

4. We believe that state agencies (meaning governmental agencies at all levels) that provide free laboratory service should confine their work to indigents, and to that immediately necessary and incident to the control of communicable diseases.

5. We believe that pathologists should limit their practice of pathology to hospital laboratories and/or laboratories personally owned by pathologists. Some exceptions might be made in the case of group medical practice provided the pathologist shares in ownership as do other members of the group.

6. We believe that the public health laboratory law under which laboratories are now registered in the State of Michigan should be revised so that only laboratories under the direction of a pathologist may be eligible for registration and that the supervision by the State should be under the auspices of the State Board of Registration in Medicine.

7. We believe that the Michigan Pathological Society should make its opinion known to the Council of the Michigan State Medical Society regarding the appointment of any Commissioner of Health.

8. We believe that at least one of the members of the State Council of Health should be appointed from the membership of the Michigan Pathological Society on recommendation of the Society.

9. We believe that the Michigan Pathological Society should set up its own standards of checking clinical laboratories and lend its assistance through advice and cooperation to the properly constituted legal authorities.

10. We believe that clinical laboratories should not be operated in hospitals or governmental institutions for other than *regular* hospital or clinic patients unless the net income so derived reverts to the pathologist of that hospital or institution.

11. We believe that the renewal certifications for a registered Medical Technologist should be endorsed by the pathologist under whose supervision she (or he) is working or if she (or he) is employed by someone other than a pathologist, that it should be endorsed by the State Councilor of the American Society of Clinical Pathologists. The supervising pathologist or the Councilor, as the case may be, shall determine, before endorsement, that the applicant has been complying with the code of ethics of the Registry of Medical Technologists of the American Society of Clinical Pathologists.

12. We recommend that all pathologists and pathological societies work and strive for the accomplishment of these beliefs.

DR. SUNDERMAN ACCEPTS POSITION AT TEMPLE

Dr. F. William Sunderman, associate professor of research medicine at the University of Pennsylvania School of Medicine, has been appointed to the staff of the Temple University School of Medicine and the Temple University Hospital, according to an announcement by Dr. William N. Parkinson, dean. Beginning July 1, Dr. Sunderman will become director of the Temple University Laboratory of Clinical Medicine and professor of clinical pathology.

Dr. Sunderman took his undergraduate work at Gettysburg College and received his medical degree at Pennsylvania in 1923. He has been a member of the medical faculty at Pennsylvania since 1925. During the war, he devoted considerable time to the atomic bomb project. The Navy Department cited him one year ago for exceptional service in research and development of RDX, picric acid and DINA. He served as medical director of the Explosive Research Laboratory, a division of the National Defense Research Commission.

Dr. Sunderman is a governor of the College of American Pathologists, a member of the American Board of Pathology, secretary of the College of Physicians of Philadelphia and a member of the editorial board of the American Journal of Clinical Pathology.

MEDICO-LEGAL CONFERENCE AND SEMINAR

The Departments of Legal Medicine of the medical schools of Harvard, Tufts and Boston University in association with the Massachusetts Medico-Legal Society will present, from October 13-18, 1947, a six-day program of lectures, conferences and demonstrations on the investigation of deaths in the interests of public safety. Attendance will be limited to 25 persons who have registered in advance.

Further information may be obtained from the Department of Legal Medicine, 25 Shattuck Street, Boston, Massachusetts.

GARGOYLISM

REVIEW OF THE LITERATURE AND REPORT OF THE SIXTH AUTOPSIED CASE WITH CHEMICAL STUDIES*

REUBEN STRAUS, M.D., REUBEN MERLISS, M.D., AND RAYMOND REISER, Ph.D.

From the Departments of Pathology and Medicine of the Cedars of Lebanon Hospital, Los Angeles, California

Within the past twenty-five years there has been recognized and accepted as a specific entity a characteristic grotesque malformation of the body believed to be associated with a lipoid dystrophy variously referred to as gargoylism, lipochondrodysplasia, Hurler's syndrome, osteochondrodystrophy and dysostosis multiplex. The disease is relatively uncommon, only about 65 cases having been reported to date, and of these cases only five have come to autopsy. Although some doubt may exist regarding the diagnosis in some of the cases not autopsied, in all probability there have been many cases which have passed unrecognized, unreported, or have been improperly classified.

REVIEW OF THE LITERATURE

As far back as 1856, a number of syndromes have been described, characterized by physical abnormalities in specific portions of the body which tended to separate them into distinct categories. Hunter,²⁷ in 1917, was the first to describe a condition in two brothers, aged 8 and 10 years, with certain generalized abnormalities which caused him to differentiate their condition from the other related conditions. Probably chiefly because Hunter's contribution was overlooked, Hurler,²⁸ who reported two additional cases in 1919, is most frequently credited with being the first to recognize this condition as a specific entity. Comparison of the various cases subsequently reported as gargoylism reveals many that are strikingly dissimilar, but when the composite picture of the syndrome is compared with an individual case, the overlapping features make the relationship readily apparent.

The disease affects individuals of either sex and is about 35 per cent more frequent in males than in females. Although the physical and developmental abnormalities are usually first noted in early childhood, they can be projected occasionally into the period of infancy. These persons usually die before the twentieth year from intercurrent infection or from cardiac failure complicating the thoracic deformities. A total of 31 cases (48 per cent) has been reported in twins and siblings.^{3, 11, 15, 16, 21, 23, 25, 45, 46} By far the majority of cases has occurred in the Caucasian race, only one being recorded in a Negro,²⁵ and one in a Mongolian.¹⁵ Consanguinity of the parents apparently is not significant since it was described on only four occasions.^{4, 20, 21} Because of its high familial in-

* This study was supported by a grant established by Mr. Harry Leveson of Los Angeles. Received for publication, June 16, 1947.

cidence, its occurrence in infants and children and its developmental abnormalities, the disease is universally regarded as congenital.

The different manifestations of the syndrome, culled from the previously reported cases, are characterized as follows.

These patients are usually shorter than average, some being pronounced dwarfs. Their *features* are coarse and ugly. This and the generalized physical deformities form the basis for their being referred to as "grotesque", and explains the adoption of the descriptive term "gargoylism". The *skin* is usually described as dry and coarse and occasionally as lax.^{4, 40}

The *head* is constantly large, frequently classified as scaphocephalic, acrocephalic, brachycephalic and oxycephalic with large supraorbital ridges. The ears are frequently large, relatively low in position, and retracted against the head. Deafness has been infrequently mentioned. The root of the *nose* is often depressed, the nares broad and tilted anteriorly. The *lips* are usually thick, the tongue large and the palate often highly arched. The *teeth* are poorly formed and widely spaced. The mandible is broad and heavy.

The *ocular* changes are considered highly significant. The interorbital distance is wider than average and occasionally there may be a mild internal strabismus. A very important but not constant feature^{21, 25, 37, 41} is a diffuse or spotty cloudiness of the cornea which may interfere with vision. This has been variously attributed to a corneal dystrophy^{13, 14} and to a lipid infiltration.^{22, 34, 38}

The *neck* is short and the hunching of the shoulders adds to the appearance of the head resting directly on the torso. The thorax is large and appears fixed in a position of deep inspiration. A pronounced dorso-lumbar kyphosis is described as a characteristic but not constant feature of the syndrome.^{15, 21, 22, 26, 30, 37, 41, 46, 50}

The *abdomen* is usually large and protuberant. Umbilical and inguinal hernias are frequently present. An enlargement of the liver and spleen is often present but this has not been constant.^{4, 22, 30}

With but one exception,¹⁴ the most frequent abnormality is the flexion deformity of the *extremities*. This is characterized by a pronounced limitation of extension, but no interference with further flexion of the involved joints and is most frequent in the fingers. The larger joints such as the elbows, shoulders, hips and knees are also often involved. Coxae valga, genua valga, talipes equinovarus, pes planus and pes cavus are also not infrequently mentioned. The *extremities* have been described at times as appearing shorter than usual.

Bony changes. Roentgenographic examinations reveal widening of the suture lines, a large anterior fontanelle and occasional bony ridges. Stressed as important but not found in all cases^{26, 34, 36, 45} is the presence of an enlarged sella turcica. The kyphosis, when present, is caused by an angulated vertebral body. Other vertebral bodies are delicate and narrow especially in the cervical region. This narrowing results in a shortening of the spine which contributes to the dwarfism.

The *ribs* usually are fixed in a horizontal position and the distal portions show varying degrees of broadening. The clavicles are often massive and heavy. The

scapulas are frequently revealed in an elevated position. The long bones and phalanges are not infrequently described as somewhat shorter and broader than usual, with delayed centers of epiphyseal ossification in some.

These bone changes are by no means constant since cases with no significant bone abnormality on roentgen examination have been described.^{15, 22, 28, 44}

Intelligence. Most of the patients are reported as being mentally defective in variable degree. A few, however, are said to have had normal intelligence and an occasional patient, intelligence greater than normal.^{1, 15, 21, 22, 25, 35, 38, 45}

Blood chemical studies have shown no constant or significant deviation from normal. In a few the blood cholesterol values were slightly to moderately elevated.^{14, 38, 39, 42}

To date only five cases have come to autopsy.^{1, 34, 41, 48} A sixth case was mentioned by Washington⁵² but no detailed report of it could be found in the literature. Four of the reported cases concerned males, and one a female. Their ages were as follows: 3, 6½, 7, 8½ and 19 years.

With the exception of Kressler's and Aegerter's case,³⁴ and with a few minor exceptions in some other cases, the thoracic and abdominal *viscera* and the *endocrine glands* presented little significant abnormality. The first of the two cases reported by Ashby and associates¹ presented a thyroid gland of the "foetal type" microscopically, and in the other case, the thyroid gland showed "moderate interstitial fibrosis". The pituitary body of the former case was also "slightly hyperplastic". In Reilly's case,⁴¹ the thyroid gland showed "slight interstitial fibrosis and hyperplasia". This case also presented focal necrosis of the anterior lobe of the pituitary gland. The spleen, but not the liver, was moderately enlarged; histologically, it was not remarkable. In another patient,¹² who did not come to autopsy, but who underwent laparotomy, an enlarged liver and spleen were found and biopsies of tissue from these organs also revealed no evidence of lipoidosis.

The case reported by Kressler and Aegerter³⁴ was the only one which presented striking visceral changes, many of which were similar to those seen in our patient. The liver and spleen were moderately enlarged. Histologically, the liver presented considerable vacuolation of the parenchymatous cells which resembled a severe fat phanerosis. A small number of vacuolated cells were seen in the spleen. Large vacuolated endothelial cells were described in the lungs. Many of the lymph nodes were enlarged and prominent nests of vacuolated cells replaced large areas of the pulp. Scattered vacuolated cells were described in the thymus, testicle, thyroid gland and pituitary gland. In places, the myocardial fibers were vacuolated. In the hematoxylin and eosin stained sections, the alteration of the tissues resembled a lipid infiltration but the areas failed to stain with the usual fat stains. No anisotropic bodies were seen with polarized light. Because of the failure to demonstrate the presence of a lipid with the usual methods, the infiltrating substance was believed to be a "conjugated lipid".

Bone was examined microscopically in only one³⁴ of these five cases and it revealed no histologic abnormality. Washington,⁵² quoting from his own unpublished case, presented histologic evidence of arrest of bone development at an

osteochondral junction. References to histologic examination of the bones of one of Hurler's cases by Pfaundler could not be verified by a search of the literature and also could not be found by Binswanger and Ullrich.⁴

Attention in all cases was centered chiefly on the *brain*. The first of Hurler's original cases was later described by Tuthill.⁴⁸ This, unfortunately, was complicated by the presence of cerebral and meningeal tuberculosis and a moderate hydrocephalus. The weight of this brain was not recorded. Of the other patients, three had brains that were heavy; one, a 3 year old, had a brain weighing 1077 gm.; another, an 8½ year old, had a brain weighing 1200 gm.; and the third, a 6½ year old, had a brain weighing 1700 gm. A fourth patient, 19 years of age, had a brain weighing 1043 gm.

With the exception of one case,⁴¹ the histologic examination of the brains was especially stressed. There was a "ballooning" of the large ganglion cells of the cerebral cortex, basal ganglia, cerebellum, pons and medulla oblongata. This was most pronounced in the basal ganglia and in the olivary bodies. In these cells there was a striking accumulation of a granular brown pigment with an eccentric displacement of the nucleus. Some of the cells presented degenerative changes such as pyknosis, karyolysis and loss of Nissl substance.

In the cytoplasm of these ganglion cells, a relatively large amount of lipoidal substance was demonstrable with the usual histochemical fat stains, chiefly sudan III and IV. Ashby *et al.*¹ demonstrated that this material was soluble in some, but not all, of the usual fat solvents. In three cases,^{1, 34} the tissue was examined with polarized light and in only one instance¹ were anisotropic granules found. In two of the cases^{1, 48} a small amount of lipoid material was noted in the neuroglia of the white matter and in the adventitia of some of the small blood vessels. Tuthill⁴⁸ also described focal areas of demyelination but this was not seen in the other cases.

Because of the intracellular lipoidosis in the brain of these patients and the coexistent mental impairment a similarity was drawn between gargoylism and the primary idiopathic lipid dyscrasias, chiefly, Niemann-Pick's disease. It was believed that if the character of the infiltrating lipid could be determined and proved to be a cerebroside, sphingomyelin or cholesterol, the relationship to one of the lipid dyscrasias could be established. Gross chemical examination of the brain was attempted in only one case.¹ (We exclude the case reported by Jervis³⁰ and quoted by others, because of the absence of certain essential features of gargoylism; by careful differential diagnosis the author also concluded it was an entity separate from gargoylism.) Except for a moderate decrease of cerebroside in the cortex of the brain, no other abnormality was found. This will be discussed further in comparison with our own analysis.

REPORT OF CASE

Clinical History

The patient, A. C., a 29 year old white, unmarried male, of Jewish parentage, was admitted to Cedars of Lebanon Hospital with the provisional diagnosis of atypical pneumonia and multiple unexplained deformities. The physical exami-

nation, x-ray findings and clinical course confirmed the impression of virus pneumonia. Immediate treatment was directed toward the pneumonia, recovery from which occurred in about ten days.

Attention was then concentrated on an explanation for the physical deformities. The patient was the oldest of three brothers. The brothers were living, in good health and had well-proportioned, handsome features (Fig. 3). The mother, aged 47 years, was living and well. The father, a musician, committed suicide in a fit of depression at the age of 40 years. There was no history of consanguinity or of relatives with physical deformities on either the maternal or paternal side of the family. One paternal aunt was known to have periods of insanity.

The patient was born about two or three weeks after the expected date and delivery was instrumental. He weighed ten and one-half pounds at birth and appeared entirely normal. At the age of 3 months, he had a convulsion for no obvious cause. There was an episode of fever and diarrhea at the age of 1 year. At the age of 4 years, he had an attack of whooping cough, and at 8 years of age, an attack of measles. When about 9 years old, he had an attack of acute pharyngitis for which he was given diphtheria antitoxin, and following which he developed symptoms of serum sickness. In all other respects the patient had been in good health (Fig. 1).

Soon after the last-mentioned illness the mother noticed that the child had developed a peculiar gait and a tendency to walk on his toes with his feet turned out, legs far apart and the left leg lagging. This abnormality appeared to have developed gradually. A physician was consulted who made a diagnosis of old poliomyelitis with bilateral pes cavus, more severe on the left side. At that time it was noted that neither foot could be flexed above a right angle. At the age of 11 years the patient was operated on; the plantar fascia of each foot was incised, the Achilles tendon lengthened, and the tendon of the flexor hallucis longus sutured to the first metacarpal bone. At the time of this operation routine physical examination revealed no other further abnormality. The operation¹ failed to result in improvement.

The child continued at school and was able to walk, but with some difficulty. He required special shoes to fit his deformed feet (Fig. 2).

At the age of 12, he became aware that he was unable fully to extend the four fingers of each hand. Although he could flex his fingers at will, they gradually underwent a flexion contracture of a severe degree with inability at extension. The thumbs were uninvolved. A year later the child first noted inability to fully adduct the thighs although further abduction could be performed. An abduction deformity also gradually developed to a severe degree. At about this time inability to completely extend the elbow and knee joints became apparent.

Gradually over a period of years a definite change developed in his entire body structure. His head became disproportionately large and the forehead more prominent. He lost the ability to elevate his eyes above the horizontal but there was no interference with vision. His shoulders became hunched, his back rigid, and his abdomen protuberant.

His grotesque appearance finally forced him to leave high school, where he

had been an outstanding student. He became sensitive about his appearance, avoiding visitors and doctors.

At the age of 18 he developed an acute suppurative appendicitis with perforation of the appendix and peritonitis. The appendix was removed and abdominal drainage was instituted. This was followed by a large incisional hernia which necessitated repair one year later.

When the patient was about 24 years of age, it was noted that his chest appeared enlarged and that his finger tips were clubbed. His abdomen was distinctly more protuberant and the incisional hernia redeveloped. He then complained of pain in the back. Owing to the contracture of his extremities he found it increasingly difficult to walk and finally was forced to use both hands and knees for locomotion. This resulted in large calluses of the fingers and knuckles (Fig. 6).

About one year before his admission to this hospital, he began to have dyspnea on exertion and shortly thereafter cyanosis of the lips and finger tips. Soon after this there developed edema, first of the scrotum and then of the lower extremities. The veins of the abdomen became very prominent.

Through all this the patient was an intelligent and voracious reader, interested in world affairs and an intense lover of music. He played chess and gained recognition, locally, as an outstanding player. Although deformed, he strongly desired to live.

Physical Examination

The *head* of the patient appeared relatively large, and the face broad. The frontal bones and supraorbital ridges were very prominent. The scalp was thrown into longitudinal folds, the hair was relatively coarse and the skin of the face was of coarse texture (Fig. 5). The *eyes* were wide-set and there was inability to raise them above the horizontal level. The corneas were clear, convergence was poor, vision was normal and the fundi showed normal discs and retinas. Slit lamp examination of the corneas revealed no opacities. The *ears* were low in position and retracted against the skull. There was no deafness. The lips appeared thick and prominent. The teeth were spaced far apart. The tongue appeared broad but not thickened and the palate had a high arch.

The veins of the *neck* were quite dilated and prominent, but no pulsations were present. The neck was short and completely rigid. The head seemed to rest directly on the shoulders. The thyroid gland and the cervical lymph nodes were not palpable.

The *chest* appeared to be fixed in deep inspiration. The shoulders were hunched. Breathing was entirely abdominal and the subcostal angle was wide. The diaphragm was elevated on both sides. The heart was of average size, the rate was normal, the rhythm regular, and no murmurs were heard. The blood pressure was 125/75 mm. Hg.

The *abdomen* was large and distended, with bulging in both flanks. There was pitting edema of the anterior and lateral aspects of the abdomen. Numerous large veins were present. The lower edge of the liver was palpable 8 cm. below the right costal margin in the midclavicular line. The spleen was palpable



FIG. 1. Patient at the age of 9 years.

FIG. 2. Patient at the age of 14 years. Note hands, shoulders and feet.

at the costal margin. There was a slight umbilical hernia and a large, bulging, incisional hernia. A fluid wave and shifting dulness were present.

The *back* was perfectly straight and rigid with loss of the usual dorsal and lumbar curvatures. There was no kyphosis and no tenderness.

The external *genitalia* showed marked edema, chiefly of the scrotum. The testicles were small but in normal position. Rectal examination revealed a few hemorrhoidal tags and a small prostate.

The *extremities* were greatly deformed. The thighs were fixed in about 45° abduction. The knees presented about 80° flexion with 15° of motion remaining.



FIG. 3. Patient at the age of 24 years with his two brothers.

There was marked bilateral pes cavus and talipes equinovarus deformity of about 120°. Marked pitting edema was present in the lower extremities up to the inguinal regions. Operative scars were noted on the plantar aspect of both feet. The patient could not raise his arms above an 80° angle with his body. The right elbow was flexed to an angle of about 110°, the left to an angle of 170°, and there was stiffness of his wrists. The hands were "claw-shaped" due to marked flexion contracture of all fingers except the thumbs. Pronounced cyanosis and clubbing of the finger tips and calluses over the knuckles were present. The musculature of all extremities was moderately atrophic.

A neurologic examination revealed normal reflexes.

Subsequent Course

A diagnosis of gargoylism was suggested by Dr. Arthur Hoffman, attending physician in medicine, and comparison of the features of this case with those already reported in the literature confirmed this impression.

Therapy was directed toward the anasarca and dyspnea; treatment with paracentesis, mercurial diuretics and intravenous plasma resulted in moderate improvement. He was discharged after four weeks to the care of a rest home. About one month later he was readmitted with dyspnea, cyanosis and edema which grew progressively worse. Signs of consolidation were found at the base of each lung. On the third hospital day the patient suddenly became irrational and expired after a few hours. Death appeared to be due to anoxia produced by the limited vital capacity and impaired venous return to the heart, which was further aggravated by the anasarca and a bronchopneumonia.

Laboratory Findings

There were no significant findings in the blood counts or in the urine. Urinary creatine was absent. Blood cholesterol ranged from 164 to 185 mg. per 100 cc. Total blood proteins varied between 6.3 and 7.4 gm. per 100 cc., but 50 per cent of the total consisted of globulin. The icterus index was elevated to 17 units. Bromsulfalein elimination test revealed impaired liver function, 80 per cent not being removed from the blood stream. The other blood chemical determinations were not abnormal. Electrocardiographic studies and blood circulation times were normal. Venous pressure was recorded as 210 mm. of water. The vital capacity was reduced to 700 cc.

Roentgenographic Examination

Roentgenographs were taken of the entire skeleton. The suture lines of the skull were slightly wider than normal; the cervical and thoracic vertebrae were more delicate, narrower and smaller than average (Fig. 4); the ribs appeared fixed in a horizontal position and the lower ones were broader than average; there was a widened mediastinal shadow; the pelvis was funnel-shaped. Except for slight osteoporosis of disuse, the bones and joints showed no abnormality.

AUTOPSY FINDINGS

Gross Examination

The autopsy was performed twelve hours after death. The following description will be limited to the positive findings and the significant negative items and will exclude major external distortions described above. Some of these features are well exemplified in the photographs. The body was dwarfed, measuring 56 inches in length, but allowing for the flexure of the hips and knees it measured 61 inches. It weighed an estimated 140 pounds, part of which was due to ascites and edema.

The muscles, for the most part, were fairly well developed. Those of the anterior abdominal wall were considerably hypertrophied, but the muscles of



FIG. 4. X-ray film showing narrow vertebral bodies.

the extremities were atrophic in striking contrast. Unduly large blood vessels were present in the intermuscular and fascial planes.

The calvarium was of average thickness and cut with the usual amount of resistance. The base of the skull presented nothing remarkable. The spine was straight and the vertebral bodies were relatively small and delicate. Section through one of the vertebral bodies showed no gross abnormality; the regional intervertebral cartilage also showed nothing remarkable. The anterior longitudinal ligament over the segment of vertebra that was removed was strikingly thicker than average. The pelvis was small and shallow.

The heart weighed 310 gm. but presented no abnormality. The left lung weighed 520 gm. and the right 720 gm. There was extensive bronchopneumonic consolidation of the lower lobe of each lung and diffuse congestion and edema. Located chiefly in, and just below the pleura, and also deep in the parenchyma in a few places, were numerous white, firm nodules of fine texture, 1 to 3 mm. in diameter.

The spleen weighed 1030 gm. The capsule was slightly thickened; the splenic follicles were numerous and slightly more prominent than usual. The pulp was severely congested and firm. The lymph nodes presented the most striking abnormality. Practically all of the thoracic and abdominal lymph nodes were markedly enlarged, some measuring 3 cm. in largest diameter. The majority were discrete, while others were matted together. The nodes were firm, mottled, light and dark gray to pinkish red and presented a coarse texture on section.

There was approximately 2 liters of amber color cloudy fluid in the peritoneal sac. The gastro-intestinal tract presented no appreciable abnormality except for a solitary grayish white nodule, 2 cm. in diameter, located in the duodenum and similar to those seen in the lung. The liver weighed 2040 gm. and presented extensive portal cirrhosis with nodules of hyperplasia up to 3 cm. in largest diameter.

The kidneys were fused at the lower pole to produce a horseshoe-shaped kidney with a double renal pelvis. The cut surface of the parenchyma was not remarkable. The internal genitalia were not abnormal grossly.

The brain weighed 1420 gm. The external aspect and the surfaces made by repeated coronal sections presented no abnormality grossly. Section of the brain stem also presented no gross abnormality.

Histologic Examination

The tissue was fixed in 10 per cent formalin, embedded in paraffin and sectioned at 6 microns. One hundred and twenty-seven routine sections were stained with hematoxylin and eosin. Eighty additional sections of selected pieces of tissue were stained with scharlach R and osmic acid, Weigert's elastic tissue stain, and Masson's trichrome stain. About ten unstained sections were examined with polarized light. Thirty-four sections were treated with fat solvents and acid and basic dyes.

Lymph nodes. The most striking histologic changes were found in the lymph nodes; 94 sections were examined. In most of the lymph nodes the normal

architecture was replaced in part or in whole by alveolated masses of large mononuclear cells with abundant cytoplasm which, for the most part, was vacuolated but was in some part granular (Fig. 7).

Several sections of the lymph nodes, unstained, mounted in water revealed scattered amorphous granules in the interstitial stroma and within some of the large mononuclear cells. These granules varied from 1 to several microns in diameter, were greenish brown and slightly refractile. Superficially they resembled anthracotic pigment. Cold absolute alcohol, xylol, benzene, acetone, dioxane and chloroform did not dissolve these granules. Hot benzene, ether, dioxane, chloroform, xylol, alcohol and acetone caused them to completely disappear. Control lymph nodes with abundant anthracotic pigment were similarly treated but showed no dissolution of the pigment granules. When examined with polarized light no anisotropic granules were identified.

Sections of the lymph nodes stained with scharlach R and osmic acid revealed varying amounts of globular and granular stainable lipoidal pigment in the cytoplasm of the large mononuclear cells and in the interstitial stroma at the site of the above described granules. The amount of lipid demonstrated in this way, however, did not parallel the degree of vacuolation of these cells. With the scharlach R, the intensity and hue of the stain was not identical with that found in the neutral fat. Sections of normal lymph nodes, used as a control, revealed no evidence of lipid with these stains.

Following the procedures used by Ashby *et al.*,¹ sections of the lymph nodes were treated with various acid and basic dyes. Sections stained with acid dyes such as acid fuchsin, brilliant green, congo red, eosin, light green, orange G and Van Gieson stain revealed no coloration of the granules or the vacuolated mononuclear cells. Additional sections treated with basic dyes revealed certain changes. With Krajian's mucin stain a distinct magenta color appeared in the cytoplasm of some of the large vacuolated cells but the granules were unchanged. Sections treated with neutral red revealed a reddish pink coloration of some of the vacuoles and granules. With cresyl violet the granules developed a slight reddish cast, with thionine and toluidine blue a slight lilac color, with safranin a slight orange-yellow cast and with methyl violet no color developed. The vacuolated cells were unstained by these dyes. Several sections of lymph node treated with Lorrain-Smith stain revealed a blue discoloration of the granules but not of the vacuoles. (According to Mallory, this is characteristic of cerebro-sides.) The control lymph nodes including anthracotic pigment and regional fat similarly treated showed no affinity for these stains.

Sections of the *lungs* presented considerable bronchopneumonia. The white nodules described in the gross consisted of alveolated groups of large mononuclear cells similar to those seen in the lymph nodes (Fig. 8). With scharlach R and osmic acid they, too, revealed the presence of lipid in the cytoplasm of some of these cells. No anisotropic granules were identified.

The nodule in the *duodenum* revealed similar vacuolated mononuclear cells which contained a small amount of lipoidal material. In addition to this, the ganglion cells of the myenteric plexus and some of the regional connective tissue

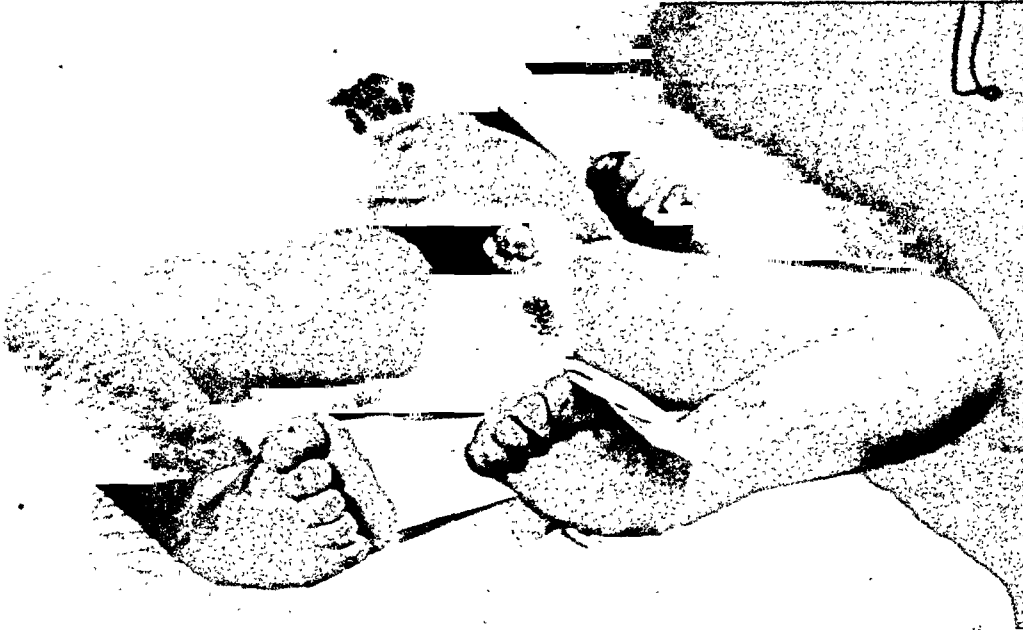


FIG. 5. POST MORTEM. Note coarse texture of skin of face, widely spaced eyes, broad nares, thick lips, retracted ears, short neck and large chest.

FIG. 6. POST MORTEM. Note large calluses of the knuckles, contractures of the fingers, incisional hernia, edema of the external genitalia and lower extremities, the pes cavus and talipes equinovarus.

cells also contained a small to moderate amount of lipid. No anisotropic granules were seen.

Sections of the *liver* presented the usual distortion of the anatomic lobules, fibrosis, and evidence of nodular regeneration, characteristic of portal cirrhosis.

The *spleen* showed moderate pulp hyperplasia, focal hemorrhage, and a few scattered large mononuclear cells, containing a small amount of lipid, similar to those in the lymph node, lung and duodenum. The thymus also contained a few scattered mononuclear cells of a similar nature.

The *kidneys*, *pancreas*, *prostate gland* and *testicles* presented nothing remarkable except for absence of active spermatogenesis. When stained with scharlach R, a small to considerable amount of lipids could be seen, particularly in the epithelium of the convoluted tubules of the kidneys, in the glandular epithelium and stromal cells of the prostate, in the interstitial cells of Leydig, the stromal cells and in the epithelial cells of the testicles, and in the acinar cells and islands of Langerhans of the pancreas.

The *pituitary gland* showed a slightly enlarged anterior lobe. Some of the chromophobe cells were moderately vacuolated. The other structures were not remarkable. This organ was not subjected to special stains since all of it was embedded in paraffin.

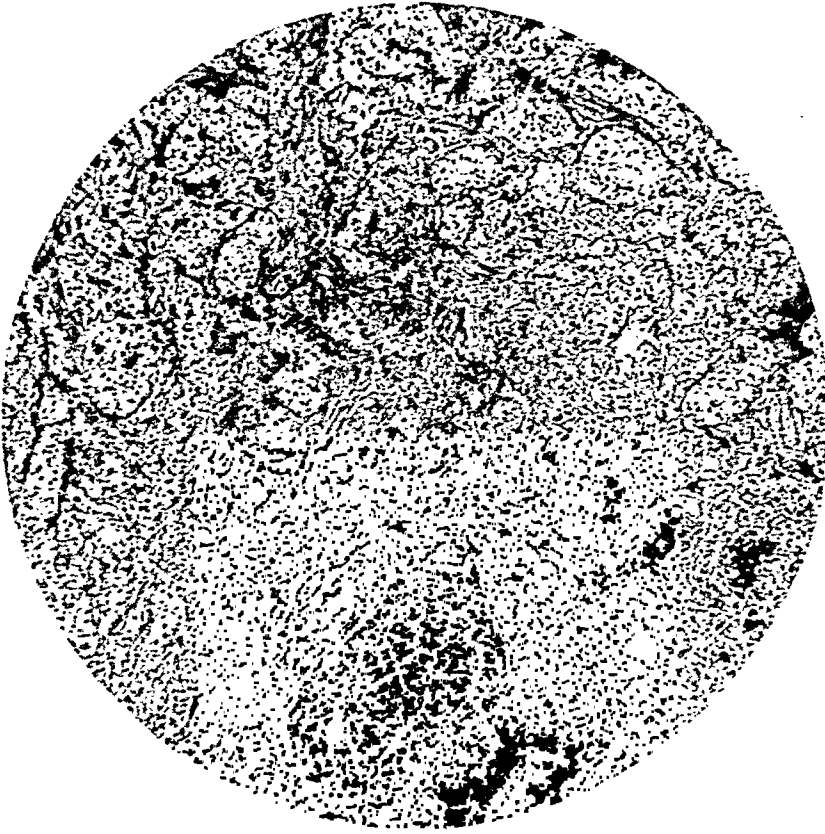
Sections taken from various portions of the *brain* showed slight variation in size of the ganglion cells. Some were small and pyknotic, others were larger and presented a moderate amount of brown pigment in the cytoplasm. The nuclei of the latter cells were eccentric due to the presence of the pigment. The pigment, however, was insufficient to cause "ballooning" of these cells. The pigmentation was least pronounced in the ganglion cells of the cerebral cortex, and most pronounced in those of the basal ganglia and pons. In places there was perineural and perivascular parenchymatous vacuolation. A small amount of brown pigment was found in the adventitia of some of the blood vessels. Additional sections of the medulla oblongata and the cervical portion of the spinal cord showed prominent ganglion cells in the gray matter, some of which appeared considerably swollen with brown granular pigment. The remainder of the tissue was not remarkable.

Sections from the cortex of the brain, the basal ganglia, pons, cerebellum and from the medulla oblongata stained with scharlach R revealed variable amounts of orange-red globular and granular lipid in the large ganglion cells. The ganglion cells of the cortex presented the least concentration of lipid, while those of the basal ganglia and of the medulla oblongata presented the greatest. In addition, a small amount of yellowish red, globular and granular lipid was noted in the adventitia and endothelium of some of the small blood vessels. No anisotropic granules were found in this tissue.

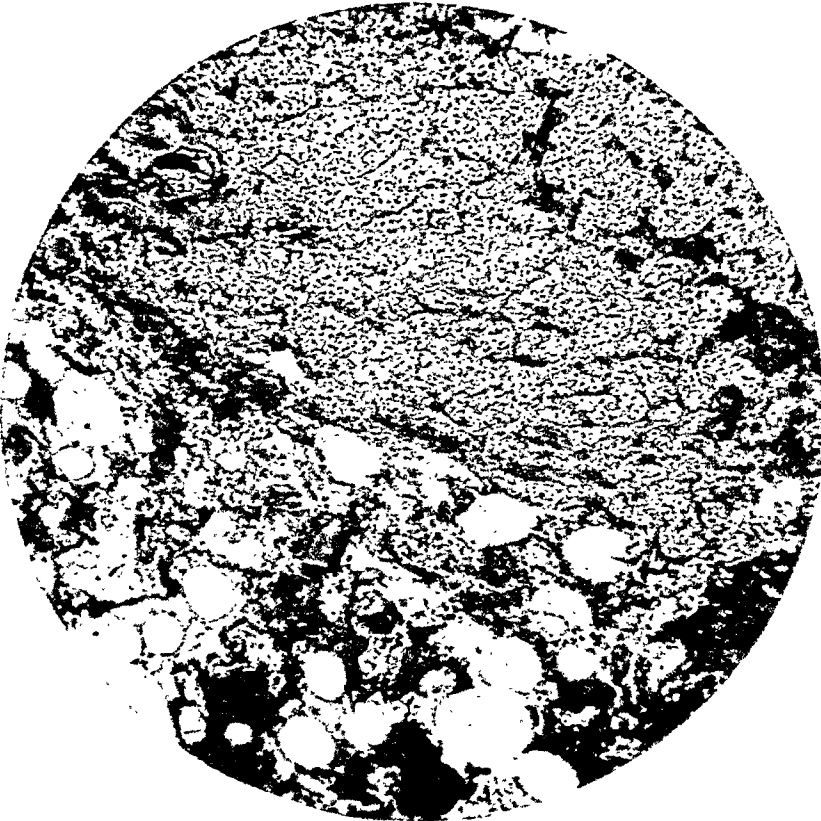
Sections of the *skin*, *muscle*, *bones*, *heart*, *thyroid gland* and *suprarenal gland*

FIG. 7. LYMPH NODE. Note normal lymphatic tissue at bottom and replacement of this elsewhere by large vacuolated mononuclear cells.

FIG. 8. LUNG. Note replacement of lung parenchyma at top by large vacuolated mononuclear cells. The parenchyma at bottom shows moderate edema.



7



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presented no appreciable histologic abnormality. The section of skin taken from the foot was markedly edematous. The periosteum and ligament on the body of one of the vertebrae was extremely thick and dense. When stained with scharlach R, no abnormal lipid deposits were noted.

ANALYSIS OF TISSUES FOR LIPIDS

Since a lipid was demonstrated in the tissues by histochemical methods, and since a relationship to the lipid dystrophies was suspected, it was deemed advisable to make a chemical analysis of the tissues in an effort to determine the character of the infiltrating lipid. The chemical determinations in this case were made by Dr. R. Reiser.

Methods

Extraction. All the tissues were formalin-fixed. Blocks of the fixed tissue were wiped dry, cut as fine as possible with a fine scissors, and ground in a mortar. About 5 gm. of the ground tissues was used for moisture determination and exactly 5 gm. of brain, 10 gm. of liver, 15 gm. of spleen, and 10 gm. of lymph nodes were used for the lipid determination.

The ground tissues were refluxed in 100 cc. of a 3:1 alcohol-ether mixture for thirty minutes and the extracts decanted in 250 cc. volumetric flasks. The extraction was repeated with 50 cc. of alcohol-ether and then with 50 cc. of ether. The dried extracted tissues were transferred to a mortar, ground until powder fine and extracted twice more with 50 cc. portions of a 3:1 alcohol-ether mixture as before. After cooling, the flasks were filled with the alcohol-ether mixture. The extracts were filtered and kept in glass-stoppered bottles in the refrigerator.

Cholesterol. Two cc. aliquots of the extracts were evaporated to dryness, the lipid extracted with chloroform and total cholesterol determined by use of the Liebermann-Burchard reaction according to Bloor.⁵

Lecithin and cephalin. These phospholipids were determined together as the moist ether-soluble phosphorus of the acetone insoluble lipids. The phospholipids were precipitated from 2.5 cc. of brain and 5 cc. of liver, spleen and lymph node extracts by evaporation of the extract to dryness in 15 cc. centrifuge tubes. Three drops of saturated strontium chloride in 95 per cent alcohol and 7 cc. of acetone were added to the dry extract. The mixture was chilled in ice-salt mixture for one hour and centrifuged. The centrifugate was washed three times with moist ether and phosphorus was determined in the evaporated combined-ether extracts by the method of Fiske and Subbarow.¹⁹ The amount of lecithin plus cephalin was considered to be 25 times the amount of phosphorus.

Sphingomyelin. This phospholipid was determined as the moist ether-insoluble phosphorus of the acetone-insoluble lipids after the method of Kirk,³³ except that phosphorus was determined by the method of Fiske and Subbarow. The factor 26 was used to convert phosphorus to sphingomyelin.

Cerebrosides. Cerebrosides were determined by a modified Kemmelstiel procedure as described by Kirk.³²

Total lipids. Total lipids were determined gravimetrically. Ten cc. of the alcohol-ether extracts was evaporated to dryness, re-extracted with ether, and dried to constant weight.

"Rest" lipid. This fraction consisted of triglycerides, free fatty acids and the fatty acids of cholesterol esters. It was obtained by the difference between the total lipids and the sum of the lipids determined individually as described.

TABLE 1
LIPIDS IN A CASE OF GARGOYLISM

	PER CENT BY WEIGHT OF DRY TISSUE			
	Liver	Spleen	Brain	Lymph Nodes
Total fat.....	12.9	12.4	50.3	40.7
Cephalin and lecithin.....	3.6	3.4	17.9	3.2
Sphingomyelin.....	0.5	0.8	6.9	0.6
Cerebrosides.....	1.1	0.7	11.4	1.1
Cholesterol.....	2.0	2.4	13.1	1.8
"Rest" lipid*.....	5.7	5.2	1.2†	34.0

* Obtained by difference.

† Value is questionable, probably due to sum of errors of other determinations.

TABLE 2
LIPIDS IN NORMAL TISSUES

	PER CENT BY WEIGHT OF DRY TISSUE						
	Liver		Spleen		Brain		Lymph Nodes
	(a)	(b)	(b)	(b)	(c)	(d)	(e)
Total fat							21.0
Cephalin and lecithin	11.7	9.43	7.70	25.23	24.8	29.6	8.9
Sphingomyelin		0.38	0.86	5.66			
Cerebrosides					5.65	11.3	0.47
Cholesterol	0.9				15.6	20.9	1.4
"Rest" fat*	2.6						10.3

* Fat is by difference (chiefly triglycerides).

(a), values by Yasuda;⁵³ (b), values by Thannhauser;⁴⁷ (c) values by Erickson;⁴⁷ (d), Values by Bodansky;⁶ (e), values in our patient.

Results

The results of the analyses of the tissues in this case of gargoylism are given in Table 1, and may be compared to Table 2 in which are given the lipid analyses of normal human brain, liver and spleen as found in the literature. No lipid analyses of normal lymph nodes were found in the literature. Therefore, lymph nodes with no apparent lesion were secured from an autopsied case and were subjected to the same analysis to serve as a control for the case of gargoylism. These results are also recorded in Table 2.

In brain, liver and spleen no marked change in any lipid constituent was found. In brain, the phospholipids tend towards a low normal and the cerebroside to a high normal. In liver, phospholipids are low and "rest" lipid high.

The lipid composition of the lymph nodes in gargoylism differs from the normal mainly by a decrease in total phospholipids from 8.9 to 3.8 per cent and by an increase in "rest" lipids from 10.3 to 34.0 per cent. (All adhering adipose tissue had been carefully removed under a hand lens.) There is, also, a relatively great percentage increase in cerebroside but the value is so low (1.1 per cent) that its significance must remain questionable. Total cholesterol remains essentially unchanged. These findings are discussed below.

DISCUSSION

From a consideration of the various case reports it appears that gargoylism has been universally accepted as an entity of congenital origin, familial in nature, due to an abnormality in growth of cartilage and bone, associated in some way with a lipid dystrophy related to Niemann-Pick's disease. It appears to us that evidence exists to cast doubt on some of these assertions.

The confusion regarding this syndrome is reflected in the multiplicity of terms applied to it. It would seem that the term gargoylism is preferable to the others in popular use, such as dysostosis multiplex, osteochondrodystrophy, lipochondrodysplasia and Hurler's syndrome, on the grounds that it is more descriptive, generic and uniformly applicable, because the syndrome was first described by Hunter and not by Hurler, and because the histogenic terms infer changes that are not always present.

The syndrome has been accepted as a congenital disease chiefly because it is a disease of children. Some^{3, 36} have suggested that it must be due to a fault in a gene. Based on the experiment of Bagg and Little, Engel¹⁶ has offered an intriguing theory according to which, in embryonic life, blebs of cerebrospinal fluid escape through the foramen anticus from the medullary tube into the subcutaneous tissue and interfere with normal growth of primordial structures with which it comes in contact. Engel also explained the lipid dystrophy on similar interference with the normal growth of the pituitary hypothalamic mechanism which controls lipid metabolism. This seems to be difficult to accept. If there was but a simple mechanical interference with embryonic growth, it would be logical to expect anatomic evidence of this interference at birth. Since the disease may not become evident until several months or many years have elapsed, it is just as likely that a postnatal influence, or possibly some extraneous influence during fetal life, could be the basis for the abnormality. The high familial incidence of this disturbance, however, (48 per cent) among twins and siblings places the weight of the evidence in favor of a congenital factor as the cause.

Physical Abnormalities

Certain of the physical abnormalities appear to be constant, *viz.*, dwarfism, large head, short neck, coarse, ugly features, flexion deformities of some or all joints, large thorax and protuberant abdomen. Other features that are highly

significant but not constant are: cloudy corneas, kyphosis, enlarged liver, enlarged spleen, umbilical and inguinal hernias, mental retardation, long bones that are shorter and broader than usual, narrow and deformed vertebral bodies and lipoidosis of various organs.

The evidence, slight as it is, for an abnormality of bony growth was secured almost entirely from roentgenographic studies. Histologic examination of bone was made in only three cases. It is highly significant that only one case revealed an abnormal osteochondral junction. The bone in the other two cases was histologically normal. Since the histologic observations are divided on the subject, it cannot be definitely stated that an osteochondrodystrophy is not the basis for the bony abnormality but, on the other hand, since cases without deformities of bone do exist, the osteochondrodystrophy cannot be the universal basic factor for the production of the physical deformities.

Based entirely on inference, it has been suggested also that a lipoid infiltration of the bone marrow, similar to that in Gaucher's disease, could account for the osteodystrophy, but this has been excluded by the failure to observe such lipoid infiltration in the three cases previously mentioned.

Metabolic influences appear to have been excluded fairly definitely as an etiologic factor for the development of the abnormalities, based on clinical studies, roentgenograms and tissue examination. Although there are some features in gargoylism, such as cretinism, that superficially suggest a glandular deficiency, histologic examination of tissues has revealed no significant changes, and replacement therapy has been without effect on the progress of the disease.

One of the constant features of the syndrome that appears to have received insufficient general attention, but was emphasized as distinctive by Ashby *et al.*¹ and Lurie and Levy,³⁷ is the presence of the flexion contractures of the joints. Although usually interpreted as an abnormality of the articular cartilage of the joints, it appears that the key to the understanding of most, if not all, of the deformities lies in this abnormality. The basic clue for this theory exists in the similarity of the "claw-like" flexion deformity of the hands in gargoylism to that of Dupuytren's contracture.

Application of this concept of fascial contractures to the other physical deformities of gargoylism appears relatively simple. The bilateral pes cavus could be due to contracture of the plantar fascia and the marked equinovarus deformity to contracture of the ligaments of the ankle, including the cruciate ligament. The abduction of the thighs could be due to contracture of fascia lata or other ligaments about the head of the femur; the flexion of the knees to the contracture of popliteal fascia or ligaments of the knee joints; and rigidity of the spine to contracture of the anterior and posterior longitudinal ligaments, the ligamentum flavum and possibly the interspinous ligaments, especially since there is no bony union to explain the rigidity. Inability to fully abduct the arms could be due to a contracture of the axillary fascia; flexion of the elbows to contracture of the fascia of the cubital fossa; the fixed thoracic cage to contracture of the intercostal and deep thoracic fascia; elevation of the scapulas to contracture of the fascia extending between the scapula and the ligamentum nuchae; and the furrowing of the scalp could be due to contracture of the galea aponeurotica.

It seems that disease of collagen, chiefly of fascia and ligaments, is a more adequate explanation for the physical deformities than any previously considered. Some evidence for its existence, however inadequate, is found in the unusually thick fascia of the abdominal wall and that overlying the spine as noted in our case. Examination of the fascia of the other joints was not made since this concept did not occur to us until long after the autopsy was completed.

It is possible, but by no means proved, that normal bony growth could be interfered with in two ways by this contracture of fascia. Circumscription of bones by abnormally rigid fascial and tendinous structures could limit its growth, and unequal tensions could produce deformities. On the other hand, since bone and cartilage are mesodermal derivatives, it is also conceivable that they could be directly influenced in the process of formation by the same factors influencing the development of fascia. Since the disease has a variable onset, and since the susceptibility of bones to extraneous influences is variable at different ages, the pleomorphic picture of the skeletal abnormalities could then be explained.

Neuromuscular disease as the basis for the contracture deformities is readily excluded by the normal reflexes and the ability actively and passively to extend and flex the joints within the limits imposed by the contractures.

Lipoid Dystrophy

The concept that a lipoid dystrophy was a component part of this syndrome was introduced by Tuthill,⁴⁸ based primarily on the demonstration of an intracellular and extracellular lipid in the brain of one of Hurler's original cases. Because of this, and because the patient was mentally deficient, the disease was likened to Niemann-Pick and Tay-Sachs' disease. Intracellular lipid was demonstrable with the usual fat stains in the brain tissue of all but one of the subsequent cases in which the patients came to autopsy. The concept was further "strengthened" by the presence of the enlarged liver and spleen, the corneal opacities and the increased blood cholesterol values, all of which were interpreted as part of the picture of lipoid dystrophy.

Examination of this evidence reveals certain definite weaknesses. The increased cholesterol is an infrequent finding. The diffuse and granular corneal opacity has been studied primarily *in vivo* with the slit lamp and has been interpreted as a lipoid deposit. It also has been examined histologically and histochemically in only three cases. In one case³⁴ only empty slits were found. In the two other cases,^{2, 8} an intercellular granular pigment was found which was interpreted as a complex lipoid deposit.

The enlargement of the liver and spleen is also not constant. Two such instances^{12, 41} where tissue was examined microscopically, revealed no lipoidosis. In only two of the six autopsied cases was there widespread histologic evidence for visceral lipoidosis, but here, too, the histochemical evidence of lipid was not clearly defined. In one case,³⁴ the lipid was not demonstrable with the usual fat stains, while in our case the amount of lipid demonstrable with scharlach R and osmic acid was appreciably less than expected. This discrepancy has been explained, and perhaps correctly, as due to an alteration of the character of the

lipid by conjugation with a protein. The occurrence of cirrhosis of the liver might be accepted as further evidence for the concept of a generalized lipoidosis since it has been described in the primary idiopathic lipoidoses.³¹

The demonstration of small amounts of lipoids with the usual fat stains in unusual positions such as in the lung, kidney, intestinal wall, stromal and epithelial cells of the testicle, prostate gland and epididymis, and the large amount in the lymph nodes is evidence in favor of the presence of a lipoidosis.

As a control for the fat stains of the brain tissue of our case, several normal brains of patients of the same age group were selected and sections from these stained with scharlach R. A moderate amount of intracellular lipid was found in the same areas of these brains, as in the brain of our patient. It was then obvious that a diagnosis of lipoidosis of the brain could not be made with certainty in our case. On the other hand, it must be admitted that it is impossible to detect a slight quantitative difference in lipids by this qualitative method. As a control for the lipid identified in the case of Tuthill, and as well for the other three patients under the age of 15 years, we examined sections of the brain of a 6 year old boy with tuberculous meningitis with the same fat stains. In striking contrast, only a few ganglion cells presented an extremely small amount of lipid. It is, therefore, apparent that the presence of pronounced quantities of an intracellular cerebral lipid in those patients under the age of 15 may be acceptable as diagnostic of a cerebral lipoidosis.

Chemical analysis of the organs of the patient reported by the authors failed to reveal increased concentrations of lipids characteristic of any of the major types of lipid dystrophy. In the liver the value of phospholipids was in the range of a low normal and the "rest" fat of a high normal. The content of cephalin and lecithin, and of sphingomyelin in the spleen were low. In the brain the value of phospholipids was a low normal and of cerebrosides a high normal.

Since it has been repeatedly shown¹⁹ that fixation of tissues in formalin causes a drop in the lipid phosphorus, but no change in the total fatty acids, the above low values for phospholipids could be explained. Considering the limitations of the methods for sphingomyelin and cerebroside determinations and the changes that occur in tissues fixed in formalin, it does not appear that the variations from the published normal values in this case justify the conclusion that there is any change in the lipid composition of the brain, liver, or spleen.

In only one other case¹ was the brain analyzed for total lipid and cerebroside content. The total lipids were within normal values, while the cerebroside content of the cortex of the brain was found to be considerably reduced and that of the white matter normal. While this demonstrates a disturbance in the cerebroside concentration in one area of the brain, it also is evidence against deposition of excessive quantities of lipids in the brain.

Two lipid changes in the lymph nodes are noteworthy. One is the decrease in total phospholipid from 8.9 to 3.8 per cent. The other is the marked increase in "rest" lipids. This latter fraction, by exclusion, must be simple triglyceride esters of fatty acids, except for a possible small moiety of free fatty acids and

fatty acids of cholesterol esters. The apparent contradiction between this conclusion and certain results of the histochemical studies must lie in the common observation that histochemical demonstration of the lipids of tissues is obscured by their conjugation with proteins and carbohydrates.

This infiltration in gargoylism of the various tissues, chiefly lymph nodes, probably is best interpreted as reticulo-endothelial storage of an unidentified chemical substance in complex combination. Since the substance has not been identified as a phospholipid, cerebroside or cholesterol, identity of gargoylism with Niemann-Pick disease, Gaucher's disease and Hand-Christian-Schüller's disease is thereby excluded.

Another consideration for which there is no solution at present, is the relationship of the generalized reticulo-endothelial disease to the physical abnormalities. If it be agreed that the disturbance is a disease of collagen, and since histochemical investigation of connective tissues in many places failed to reveal the nature of the abnormality, no etiologic relationship can be established. It is to be hoped that a more careful study of the fascia of involved joints and more exhaustive biochemical analyses of the tissues from subsequent autopsies will complete the picture of gargoylism.

SUMMARY AND CONCLUSIONS

A case of gargoylism has been presented together with detailed clinical, x-ray, postmortem and chemical studies. The literature on the subject has been summarized and analyzed.

Evidence has been submitted to suggest that the deformities may be produced by a disease of collagenous connective tissue, chiefly affecting the fascia and ligaments, rather than by an abnormality of bony growth as previously accepted. It is believed that abnormalities are probably initiated by congenital factors, chiefly because of the high familial incidence of the disease.

It has been shown that lipoidosis is not a constant feature of the disease entity. When present, however, it is found in the brain and in the reticulo-endothelial system, as in other idiopathic lipid dystrophies. Chemical analysis of the tissues of our case revealed a significant increase in lipid content of the lymph nodes, but not of the brain, liver or spleen. The increased lipid was, by exclusion, simple fat probably in complex protein combination. The fact that it was not a phospholipid, cerebroside or cholesterol separates this disturbance from the other idiopathic lipid dystrophies.

No relationship was established between the genesis of the physical abnormality and the reticulo-endothelial disease.

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THE PROZONE PHENOMENON IN Rh BLOCKING SERUMS*

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Soon after the recognition of the Rh factor as a common cause of human iso-sensitization, the prozone phenomenon was described in certain Rh antiserums.^{9,13} Although the full significance of the phenomenon was not at first recognized, it was eventually established that such serums contain a mixture of agglutinating and blocking antibodies, the latter of lower titer.¹⁴ Agglutination in the first few tubes of the titration is inhibited by the blocking antibody, but when the serum is diluted beyond the titer of the inhibiting substance, agglutination appears.

Since the establishment of technics for the direct demonstration of Rh blocking antibodies by their agglutination of Rh-positive cells, it has likewise been recognized that zone phenomena occasionally occur when potent Rh blocking serums are tested with cells suspended in serum or albumin. Levine,¹⁰ in referring to this phenomenon, reported having encountered it as early as 1944, and in two protocols mentioned six cases in which agglutination of albumin suspended Rh-positive cells by undiluted serum was very weak or entirely absent, while appreciable titers appeared on serial dilution. He suggested that such observations are evidence of a third order of Rh antibodies which of themselves, not only do not agglutinate Rh-positive cells, but are capable of inhibiting agglutination, even in albumin, by the common variety of blocking antibodies. He cited the work of Hill and Haberman⁸ with the Coombs² test as additional evidence for the existence of such antibodies.

In testing a large number of Rh-negative bloods with Rh-positive cells in 30 per cent bovine albumin, we have encountered several with a weak prozone, and three with a prozone so strong as to completely inhibit agglutination in the first few tubes of the titration. Since references to this phenomenon in the literature are extremely sparse, it seems of interest to report these three cases, with observations on the technical conditions which affect the appearance of the prozone, and to discuss methods of eliminating it in the diagnostic test.

REPORT OF CASES

Case 1

An unmarried woman of 72, entered another hospital for treatment of severe anemia associated with a gastric neoplasm. She gave a history of having received six transfusions without reactions twenty years before. During the present admission she was typed as group O, Rh-positive, and received eight transfusions of Rh-positive blood, several of which were complicated by chills, fever and evidence of abnormal destruction of the transfused cells. At the time of

* The opinions expressed in this paper are those of the authors and do not necessarily reflect the official attitude of the United States Army. Received for publication, June 5, 1947.

the last transfusion, the compatibility of the donor's blood was checked by a cross match using serum-suspended cells, but showed no agglutination. Her cells were subsequently retyped, and on this occasion were found to be Rh-negative. Her serum was then titrated against Rh-positive cells in acacia solution (2 per cent acacia in 1.2 per cent calcium chloride, as suggested by Levine¹¹), and was found to have a high titer of hyperimmune antibodies, with a strong prozone. She subsequently received two transfusions of Rh-negative blood without incident.

Her serum, as submitted to this laboratory, gave the reactions outlined in Table 1.

Comment. The series of hemolytic reactions in this patient was due to the transfusion of Rh-positive blood into an Rh-negative patient who had erroneously been typed as Rh-positive, and who had been sensitized by transfusions twenty

TABLE 1
ANTIBODY TITRATIONS IN CASE 1

Technic used: in saline titrations, serum dilutions and 2 per cent cell suspensions were in physiologic saline; in "albumin" titrations, serum dilutions were in normal group AB serum; 2 per cent cell suspensions were in 30 per cent bovine albumin (Armour); tubes were incubated 1 hour at 37 C.; tubes were centrifuged 3 minutes at moderate speed and examined under dissecting microscope for agglutination.

CELL TYPE AND MEDIUM	TUBE NUMBER IN SERIAL DOUBLING DILUTIONS OF SERUM																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Rh ₀ (saline).....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rh ₀ (albumin).	-	-	-	-	-	2	4	4	4	4	4	4	4	3	2	1	1	-
Rh' (saline).....	4	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rh' (albumin).....	4	4	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rh'' (albumin).....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

The Diamond-Abelson slide test with whole group 0, Rh₀ blood was negative.

years before. When the patient's serum was tested with Rh-positive cells suspended in serum, the presence of a dangerous incompatibility was missed entirely, because of the prozone of the Rh₀ antibody.

Case 2

A 30 year old housewife was referred to us because of the death of her third child from erythroblastosis fetalis. Her first two pregnancies had been uncomplicated and she bore two healthy daughters. Because of postpartum hemorrhage, she had received two units of whole blood following her second delivery. There had been no reaction to either transfusion, and she had recovered without further incident. Her third pregnancy two years later ended with the delivery of a full-term male infant, who rapidly developed severe icterus and died. The necropsy findings were characteristic of erythroblastosis fetalis, with icterus gravis.

In another laboratory, the patient was subsequently found to be Rh-negative, while her husband and two living children, as well as the two donors from whom

she had received blood were Rh-positive. No Rh antibodies could be demonstrated in her serum.

In this laboratory the blood types of the patient and her family were confirmed. On a single tube test, incubating one drop of her serum with one drop of a 2 per cent suspension of group O, Rh₁ Rh₂ cells in 30 per cent bovine albumin, no Rh antibodies could be detected. Furthermore, the Diamond and Abelson slide test was entirely negative. Because of the extremely suggestive clinical history, however, the test was checked with the antihuman globulin precipitin of Coombs,² and a strong positive reaction was obtained. Titration of the serum in albumin subsequently demonstrated an appreciable titer of Rh₀ antibodies, with a prozone (Table 2).

Comment. Sensitization in this case may have been initiated by the first two pregnancies, but probably resulted from the Rh-positive transfusions. (One need scarcely mention the folly of administering a transfusion to a parturient woman

TABLE 2
ANTIBODY TITRATIONS IN CASE 2
Technic used was same as in Table 1

CELL TYPE AND MEDIUM	TUBE NUMBER IN SERIAL DOUBLING DILUTIONS OF SERUM									
	1	2	3	4	5	6	7	8	9	10
Rh ₀ (saline).....	-	-	-	-	-	-	-	-	-	-
Rh ₀ (albumin).....	-	-	3	4	4	3	1	-	-	-
Rh' (albumin).....	-	-	-	-	-	-	-	-	-	-
Rh'' (albumin).....	-	-	-	-	-	-	-	-	-	-

The Diamond-Abelson slide test with whole group O, Rh₀ blood was negative.

without first obtaining her Rh type or of following her through a subsequent pregnancy without determining her Rh status.) When a search for Rh antibodies was finally made, they were entirely missed by another laboratory, owing to the marked prozone, and they would have been missed in this laboratory, had it not been for a strong suspicion on our part.

Case 3

A 25 year old soldier was severely injured in a jeep accident while in Germany and received, without reaction, six units of whole group O blood, unclassified as to Rh type. He was later brought to Letterman General Hospital for treatment of chronic osteomyelitis which had developed in both legs. He was found to be group O, Rh-negative, and over a period of nine months was given a series of 28 transfusions of Rh-negative blood without incident other than urticarial reactions twice, and transient chills and fever on one other occasion, presumably on a pyrogenic basis.

During a survey⁵ of previously transfused patients, his serum was found to give the reactions listed in Table 3. He was subsequently given three doses of 5 cc. of Rh₁ blood mixed into his Rh-negative transfusions without incident and

and his titer of both Rh₀ and Rh' antibodies in albumin rose to 40,000, subsequently falling to 2000 with a prozone in the Rh₀ titration (Table 3). This last serum was used for subsequent observations.

Comment. In this case, hyperimmune antibodies of both Rh₀ and Rh' types were discovered several months after the last of a series of transfusions, several

TABLE 3
ANTIBODY TITRATIONS IN CASE 3
Technic used was same as in Table 1

CELL TYPE AND MEDIUM	TUBE NUMBER IN SERIAL DOUBLING DILUTIONS OF SERUM														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Initial Test															
Rh ₀ (saline).....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rh ₀ (albumin).....	3	3	4	4	4	4	4	4	3	3	2	2	1	-	-
Rh' (saline).....	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rh' (albumin).....	4	4	4	4	4	4	3	3	2	2	1	-	-	-	-
Rh'' (albumin).....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
After Stimulation															
Rh ₀ (albumin).....	-	-	-	1	2	4	4	4	4	3	2	1	-	-	-
Rh' (albumin).....	4	4	4	4	4	4	4	4	3	2	1	-	-	-	-

TABLE 4
THE PROZONE IN Rh₀ BLOCKING SERUMS
Summary

Technic used: serum dilutions were made in normal group AB serum; cells were of patient of Group 0, Rh₀, 2 per cent in 30 per cent bovine albumin (Armour); tubes were incubated 1 hour at 37 C.; tubes were centrifuged 3 minutes at moderate speed and examined under dissecting microscope.

CASE NUMBER	TUBE NUMBER IN SERIAL DOUBLING DILUTION OF SERUM																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	-	-	-	-	-	2	4	4	4	4	4	4	4	3	2	1	1	-	-
2	-	-	3	4	4	3	1	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	1	2	4	4	4	4	3	2	1	-	-	-	-	-	-	-

of which must have been of Rh-positive blood. It was only after a series of small stimulating injections of Rh-positive blood that a strong prozone appeared in the Rh₀ titration. If this last serum had been the first one examined, and if the test cells had not contained the Rh' factor, the presence of antibodies might very well have been missed entirely.

The Rh₀ titrations of the three serums used for our subsequent observations are summarized in Table 4.

EXPERIMENTAL OBSERVATIONS

In evaluating these three serums for use with the new rapid Rh typing technic,⁷ we were surprised to observe that when the titrations were centrifuged immediately at high speed, without preliminary incubation, no prozone appeared (Table 5). This observation soon was confirmed with a considerable number of Rh antisera showing a less marked prozone.

In an effort to analyze the technical factors influencing the appearance of a prozone, experiments were set up with the following purposes:

TABLE 5

EFFECT OF IMMEDIATE CENTRIFUGING ON PROZONE

Technic used was same as in Table 4, except that tubes were centrifuged immediately, without incubation.

SERUM NUMBER	TUBE NUMBER IN SERIAL DOUBLING DILUTION OF SERUM																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	4	4	4	4	4	4	4	4	4	4	4	3	2	2	1	—	—	—	—
2	4	4	4	4	4	3	1	—	—	—	—	—	—	—	—	—	—	—	—
3	4	4	4	4	4	4	4	4	3	2	2	1	—	—	—	—	—	—	—

TABLE 6

EFFECT OF TEMPERATURE ON PROZONE

Technic used was same as in Table 4, except that tubes were incubated at room temperature (22 C.) for 1 hour rather than at 37 C.

SERUM NUMBER	TUBE NUMBER IN SERIAL DOUBLING DILUTION OF SERUM																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	3	3	3	3	3	4	4	4	4	4	4	4	4	4	3	2	2	1	—
2	3	4	4	4	3	3	2	1	—	—	—	—	—	—	—	—	—	—	—
3	3	3	3	4	4	4	4	4	4	4	4	4	2	—	—	—	—	—	—

(1) To determine the effect of decreased temperature, titrations were incubated at room temperature for one hour, rather than at 37 C. (Table 6);

(2) To determine the effect of time of incubation, titrations were incubated at 37 C. for varying intervals from five minutes to two hours (Table 7);

(3) To determine the effect of cell concentration, titrations were set up with cell suspensions of varying concentrations from $\frac{1}{2}$ to 50 per cent (Table 8);

(4) To determine the effect of speed of centrifuging on the prozone, identical titrations were centrifuged at varying speeds (Table 9);

(5) To determine the importance of complement in production of the prozone, identical titrations were set up with heated and unheated serum (Table 10).

The results of these experiments, as presented in the corresponding tables, suggest that whereas cell concentration, speed of centrifugation, and presence or absence of complement have little effect on the prozone, the temperature of in-

incubation is of considerable importance, as the prozone appears promptly at 37 C., but very weakly, or not at all, at room temperature. Time of incubation

TABLE 7

EFFECT OF DURATION OF INCUBATION ON PROZONE

Technic used was same as in Table 4, except that tubes were incubated for varying lengths of time at 37 C.

TIME OF INCUBATION	TUBE NUMBER IN SERIAL DOUBLING DILUTION OF SERUM																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Serum Number 1																			
5	-	-	-	-	2	4	4	4	4	4	4	4	4	3	3	2	1	-	-
10	-	-	-	1	2	2	4	4	4	4	4	4	4	4	3	2	2	1	-
15	-	-	-	-	1	2	3	4	4	4	4	4	4	4	3	2	2	-	-
30	-	-	-	1	2	2	4	4	4	4	4	4	4	3	2	1	1	-	-
60	-	-	-	-	-	2	4	4	4	4	4	4	4	3	2	1	-	-	-
120	-	-	-	-	-	1	2	4	4	4	4	4	3	3	1	-	-	-	-
Serum Number 2																			
10	-	4	4	4	4	4	2	1	-	-	-	-	-	-	-	-	-	-	-
30	-	2	3	4	4	4	2	1	-	-	-	-	-	-	-	-	-	-	-
60	-	-	3	4	4	3	1	-	-	-	-	-	-	-	-	-	-	-	-
Serum Number 3																			
10	1	2	3	3	4	4	4	4	4	4	3	2	1	-	-	-	-	-	-
30	-	-	-	1	2	4	4	4	4	3	2	1	1	-	-	-	-	-	-
60	-	-	-	1	2	4	4	4	4	3	2	1	-	-	-	-	-	-	-

TABLE 8

EFFECT OF CELL CONCENTRATION ON PROZONE

Technic used was same as in Table 4, except for varying concentrations of cells.

CONCENTRATION OF CELLS IN ALBUMIN per cent	TUBE NUMBER IN SERIAL DOUBLING DILUTION OF SERUM SERUM NUMBER 1																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
$\frac{1}{2}$	-	-	-	-	1	2	3	4	4	4	4	4	4	4	4	4	3	3	2	1
1	-	-	-	-	1	1	2	4	4	4	4	4	4	4	4	4	3	2	1	-
2	-	-	-	-	1	3	3	3	4	4	4	4	4	4	4	3	3	2	1	-
3	-	-	-	-	1	2	2	3	4	4	4	4	4	3	3	2	2	1	1	-
5	-	-	-	-	1	2	2	3	4	4	4	4	4	2	2	1	-	-	-	-
10	-	-	-	-	1	1	3	4	4	4	4	4	2	2	1	-	-	-	-	-
25	-	-	-	-	2	3	3	4	4	4	4	3	2	1	-	-	-	-	-	-
50	-	-	-	1	2	3	3	3	2	1	-	-	-	-	-	-	-	-	-	-

appears to be of some importance, with the prozone increasing appreciably with continued incubation.

In all of the observations relating to this point, we had used the cells of a single individual (H. K.) of group O, Rh₀ (cDe of Fisher). It now remained to be seen

whether some of the previously observed variations in the prozone might not have been due to differences in the behavior of different cell types.

As might be expected, it had been found that serums from patients 1 and 3, which contained Rh' as well as Rh₀ antibodies, did not give the usual prozones when titrated against cells which contained the Rh' antigen. When a series of type Rh₀ and type Rh₂ bloods (presumably lacking this antigen) was analyzed,

TABLE 9

EFFECT OF SPEED OF CENTRIFUGING ON PROZONE

Technic used was same as in Table 4, except for variation in speed of centrifuging. Tubes were centrifuged in Adams 6-tube angle desk centrifuge (Clay-Adams Co., Inc., New York, N. Y.). Speed was calibrated in rheostat stops.

SPEED OF CENTRI- FUGING stops	TUBE NUMBER IN SERIAL DOUBLING DILUTION OF SERUM SERUM NUMBER 1																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	-	-	-	-	-	1	2	2	3	4	4	4	3	2	1	-	-	-	-
2	-	-	-	-	-	1	2	3	4	4	4	4	4	3	3	1	-	-	-
3	-	-	-	-	-	1	2	3	4	4	4	4	4	3	2	1	1	-	-
4	-	-	-	-	1	2	2	4	4	4	4	4	4	4	2	1	-	-	-
5	-	-	-	1	2	2	3	4	4	4	4	4	4	4	4	2	1	-	-

TABLE 10

EFFECT OF COMPLEMENT ON PROZONE

Technic used was same as in Table 4, except that in one titration the serum was heated at 56 C. for 10 minutes.

SERUM HEATED OR UNHEATED	TUBE NUMBERS IN SERIAL DOUBLING DILUTION OF SERUM SERUM NUMBER 3																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	1
Heated.....	-	-	-	-	2	4	4	4	4	3	2	1	-	-	-	-	-	-	-
Unheated....	-	-	-	1	2	4	4	4	4	3	2	1	-	-	-	-	-	-	-

however, an occasional specimen of blood differed from the others in failing entirely to show the prozone in these same two serums. These observations suggested that there were subgroup differences in the antigenic specificity of these cells, perhaps on the basis of the C^w of Callender and associates,¹ or the D^w of Stratton,¹² which could be differentiated by their reactions in our zoning serums, but not by the subtyping serums available to us. This aspect of the problem is at present under further investigation.

DISCUSSION

The most reliable test for Rh antibodies, in our opinion, is that of Diamond and Denton,⁵ using bovine albumin as a suspending medium for the Rh-positive cells. This test is much more sensitive than the blocking technic of Wiener,¹⁴ and in our hands gives fewer equivocal results than the slide test of Diamond and Abelson,⁴ or the "conglutination" test of Wiener.¹⁵ It is our observation,

however, that some degree of prozoning is rather common when the serums of individuals highly immunized to the Rh factors are tested by this technic and the same appears to be true when the test cells are suspended in serum or plasma. Ordinarily, although agglutination is decreased in intensity by the prozone, enough remains to indicate that antibodies are present. Occasionally, however, the qualitative test with undiluted serum is entirely negative, even in the presence of a very high titer of antibodies. We have described three such cases, and have shown that in two of them the presence of antibodies was not at first recognized.

Various technics have been used in an effort to avoid the prozone as a source of error in the test for antibodies, but none has proved entirely satisfactory. The blocking technic of Wiener should presumably be strongly positive in zoned serums (as it was in our three cases), but this test is so insensitive in detecting low concentrations of hyperimmune antibodies as to be entirely unreliable as a single screen test. Diamond⁵ has stated that his slide test will usually detect antibodies in spite of a prozone in albumin, but two of our three cases showed an entirely negative slide test when undiluted serum was tested against Rh₀ blood. Much of the danger of a false negative test may be eliminated by testing each serum through a series of dilutions. The additional labor involved in a lengthy titration of all serums is very considerable. However, if the titration is shortened to save labor, there is the danger of entirely missing antibodies in serums, as in Case 1, which showed complete inhibition through five consecutive serial dilutions.

In view of the lack of a simple, entirely reliable screen test which regularly detects Rh antibodies without troublesome prozones, it was with considerable hope that we pursued the observations that it was *heating* which produced the prozone in our titrations. While other alterations in the technic affected the prozone very little, it appeared that when antibody tests were incubated at room temperature, or were centrifuged immediately, the prozone failed to appear. This finding suggested the following simple modification of the albumin technic of testing for Rh antibodies: Mix a drop of the unknown serum in one agglutination tube with a drop of an albumin suspension of group O, Rh-positive cells and in another with Rh-negative cells. Both tubes are immediately centrifuged at high speeds for three minutes and examined for agglutination. If the cell suspension remains smooth, the test is then incubated at 37 C. for one hour and again centrifuged and examined. Agglutination of the Rh-positive cells, either before or after incubation, while the Rh-negative control remains smooth, is evidence of Rh antibodies. Although our observations have been limited by the relatively small number of zoning serums available to us, they have led us to believe that this minor alteration in technic should completely eliminate the hazards of the false negative test due to the prozone.

SUMMARY

Three cases of Rh sensitization are described in which there was a strong prozone when the serum was tested against Rh-positive cells in bovine albumin.

The technical conditions influencing the appearance of the prozone in these serums are analyzed. It is concluded that heating to 37 C. brings out the prozone since serums which were spun immediately, or were allowed to stand at room temperature, failed to show the prozone. A new modification of the bovine albumin technic of testing for Rh antibodies is suggested, incorporating immediate centrifugation to eliminate falsely negative reactions due to prozone phenomenon.

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SIGNIFICANCE OF THE ACCELERATED REACTION IN DETERMINATION OF PROTHROMBIN TIME OF DILUTED PLASMA*

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Interest in the determination of prothrombin time on diluted plasma has been heightened by the disagreement among observers as to the significance of the accelerated reaction. Shapiro and his co-workers^{10, 12} demonstrated acceleration in cases of thromboembolic disease. Peters, Guyther and Brambel⁷ based the rationale of the administration of Dicumarol in coronary occlusion upon the acceleration of prothrombin time noted by the method of Brambel.¹ Link and his group^{4, 5} observed accelerated readings in animals given single doses of methylxanthines and vitamin K. Shapiro and Richards¹¹ confirmed this in human patients given vitamin K. Sherf and Schlachman⁹ corroborated these findings in regard to the methylxanthines. Cotlove and Vorzimer,² however, were unable to find acceleration in their patients with thromboembolic disease. Gilbert, Day and Trump⁶ denied the occurrence of acceleration after the administration of methylxanthine and also disclaimed any increase in blood coagulability, using the heparin tolerance principle of de Takats.³

In view of the dissenting opinions, it was decided to review the question giving special consideration to the method used. In a previous paper,⁸ we discussed the matter of proper diluent, and showed that plasma from which only prothrombin had been removed was superior to saline. Dilution with saline was used by the aforementioned observers. With barium sulfate as the specific adsorbant in the preparation of prothrombin-free plasma, a simple method of dilution was proposed, which we felt represented a logical approach to the determination of plasma prothrombin by the one-stage technic. This paper is a survey of the results of this method applied to the question of whether acceleration of coagulation in the dilute prothrombin determination may be used as a premonitory or diagnostic sign of thrombosis or embolism.

METHOD

The method used consists essentially of Quick's one-stage procedure modified by the use of barium sulfate-treated plasma as a diluent of the plasma to be tested. The plasma diluent is prepared by the addition of 20 volumes per cent of a 30 per cent suspension of barium sulfate to pooled plasma. The mixture is incubated at 37 C. for ten minutes, centrifuged, and the supernatant plasma is poured off and utilized as the diluent. Plasma treated with barium sulfate fails to clot upon the addition of calcium and thromboplastin, but clots within three seconds after fresh thrombin is added. Additional reasons for believing that

* Received for publication, June 20, 1947.

only prothrombin is removed from this plasma were given in our earlier report^s and will not be discussed at this time. Ten per cent dilution of whole plasma with this plasma diluent was used throughout the study. Hereafter, this test will be called the "dilute prothrombin test", and the reading, the "dilute prothrombin time".

Analysis was made of 500 consecutive dilute prothrombin determinations in this laboratory from July 1, 1946 to March 30, 1947. In addition, a group of 13 laboratory workers, all young healthy adults, was chosen for normal controls (Table 1). There were 40 patients who presented special features which will be discussed below. The normal dilute prothrombin time, determined on plasma pooled from 10 of these 13 healthy adults was twenty-four seconds.

TABLE 1

PROTHROMBIN TIME OF 10 PER CENT PLASMA SAMPLES FROM NORMAL CONTROLS USING BARIUM SULFATE-TREATED PLASMA AS THE DILUENT

CONTROL CASE NUMBER	READING IN SECONDS
1	22.4
2	20.0
3	21.8
4	26.8
5	23.2
6	20.7
7	20.8
8	24.8
8	23.7
9	20.8
10	21.8
11	23.6
12	23.7
13	25.3

RESULTS

The dilute prothrombin times given in Table 1 refer in each instance to the average of three determinations on each sample. There was not more than a variation of 0.5 second in clotting time in each sample. The results show a fairly wide range of variation in normals, the lowest figure being 20.0 seconds and the highest 26.8 seconds. As a basis for consideration of the occurrence of an accelerated reaction, 20.0 seconds was accepted as the lower limit of normal.

Study of the entire series of 500 determinations disclosed only 13 instances with dilute prothrombin time below 20.0 seconds (Table 2). Only 3 of the patients in this series had thromboembolic disease: 1 patient developed phlebotrombosis and later pulmonary embolism subsequent to myocardial infarction. Another developed pulmonary embolism, the source of which was not discovered, and one patient had a postoperative phlebotrombosis. A surprising number of patients with liver disease and biliary tract disease gave accelerated reactions.

The lowest figures encountered were in Cases 5, 10 and 12. The patient in Case 5 was a 52 year old white man who entered the hospital with a diagnosis

TABLE 2

DIAGNOSIS AND PROTHROMBIN TIMES IN 13 PATIENTS WITH ACCELERATED PROTHROMBIN READINGS AMONG 500 DETERMINATIONS OF PROTHROMBIN TIME OF DILUTE PLASMA

Note that first 3 patients listed had thromboembolic disease.

CASE NUMBER	SEX	DIAGNOSIS	PROTHROMBIN TIME, IN SECONDS, OF 10 PER CENT PLASMA
1	M	Phlebothrombosis 14 days after herniorrhaphy	19.3
2	F	Myocardial infarction, subsequent phlebothrombosis and pulmonary embolism	19.7 19.6
3	M	Pneumonia left lower lobe, pulmonary embolism	19.0
4	F	Neurofibroma of spinal cord	19.0
5	M	Acute cholecystitis with empyema and bile peritonitis	17.4
6	F	Chronic ulcerative colitis, ileostomy	18.4
7	M	Encephalomalacia, mild cerebro-vascular hemorrhage	18.3
8	M	Epistaxis of 24 hours' duration	18.8
9	F	Chronic cholecystitis and cholelithiasis	19.6
10	M	Acute arsenic hepatitis (patient had received B A L)	16.5
11	F	Chronic glomerulonephritis, diabetes mellitus	17.7
12	M	Biliary cirrhosis (patient had received vitamin K)	17.5
13	M	Acute rheumatic fever (patient had received salicylates)	19.0

of subsiding acute cholecystitis. Dilute prothrombin time preoperatively was 17.4 seconds. At operation, the gallbladder was found in a mass of adhesions and there was bile peritonitis. Dilute prothrombin times postoperatively continued to be rapid, varying from 16.6 to 20.3 seconds. The patient in Case

10 had arsenic hepatitis. This patient was placed on B A L therapy, and the dilute prothrombin time showed the most rapid reading in the series, 16.5 seconds. Case 12 represents a 41 year old white man who entered the hospital with a diagnosis of biliary cirrhosis. At this time, the icterus index was 76, and the dilute prothrombin time was within normal limits, the reading being 20.7 seconds. After a bout of alcoholism, the patient was readmitted with a hypoprothrombinemia and icterus index of 128. Vitamin K was administered and the prothrombin level steadily increased until ten days after admission when the prothrombin time was accelerated to 17.5 seconds.

On the other hand, there were 27 patients with various types of thromboembolic disease whose dilute prothrombin times were normal or in the hypoprothrombinemic level (Table 3). Myocardial infarction is included as thromboembolic disease because of the known occurrence of mural thrombosis and the frequency of thromboembolic complications. In 17 patients with uncomplicated

TABLE 3

DIAGNOSIS OF PATIENTS WITH THROMBOEMBOLIC DISEASE IN WHOM DILUTED PLASMA REVEALED NORMAL OR SLOW PROTHROMBIN TIMES

DIAGNOSIS	NUMBER OF PATIENTS
Phlebothrombosis.....	2
Thrombophlebitis.....	3
Phlebothrombosis and pulmonary embolism.....	3
Pulmonary embolism following septal myocardial infarction....	1
Renal infarction from left auricular thrombus.....	1
Myocardial infarction.....	17
Total.....	27

myocardial infarction determinations of the dilute prothrombin time were made from one to forty-seven days after the onset of infarction.

DISCUSSION

From the above data, there is little support for the belief that accelerated dilute prothrombin time may be considered as a premonitory sign or as diagnostic of thrombosis or embolism. While the number of thromboembolic cases in which normal or slow dilute prothrombin times occurred was small, considerable importance can be attached to their occurrence. Only 3 of a total of 13 patients with thromboembolic disease, excluding uncomplicated myocardial infarction, showed acceleration. The acceleration which was displayed was minimal as compared to the group of patients having nonthromboembolic diseases with accelerated reactions.

The wide range of dilute prothrombin times in normal persons does not appear to be an error inherent in the test. A sufficient number of duplicate samples was tested to assure accuracy of the method. A rigid technic also helped to control the procedure. Where the three readings did not agree within 0.5 second, the entire test was repeated. The same technician performed all of the

tests reported in this series. The variation in prothrombin times of normal persons is considered as a difference in the prothrombin activity of normal plasmas. The probability exists that use of a greater number of normal controls might enlarge the range which may still be deemed normal.

Absence of acceleration in the small number of cases of myocardial infarction reported suggests that the success of Dicumarol therapy in these cases is not related to reduction in excess circulating prothrombin as proposed by Peters *et al.*⁷ The occurrence of accelerated reactions noted in the patients with non-thromboembolic disease is difficult to explain. The cause of the acceleration is unknown and must await further elucidation of the nature of prothrombin.

SUMMARY

Analysis is reported of 500 determinations of plasma prothrombin, performed by the dilute method utilizing barium sulfate-treated plasma as the diluent. Thirteen patients were encountered with accelerated reactions and only three of them had thromboembolic disease; while 27 patients with thromboembolic disease showed normal or hypoprothrombinemic readings. It was concluded that acceleration in the dilute prothrombin reaction was not suggestive evidence of a tendency to the occurrence of thrombosis or embolism. The accelerated dilute prothrombin determination, therefore, cannot be used as a predictive or diagnostic sign of thromboembolic disease.

The cause of acceleration in the dilute prothrombin determination is not known. Its frequent occurrence, however, in early disease of the liver was noted.

Acknowledgment: The technical help of Miss Rose Mary Teresi, M.T. (A.S.C.P.), is sincerely appreciated by the authors.

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VARIATIONS IN PROTHROMBIN AND ANTITHROMBIN IN PATIENTS WITH THROMBOSING TENDENCIES*

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The advent of anticoagulant therapy in the treatment of thromboembolic diseases has brought about a particular interest in the study of blood coagulation in patients with thrombosing tendencies. The possibility of some quantitative or qualitative change in the factors involved in the coagulation process cannot be ignored. Shapiro⁶ has reported that hyperprothrombinemia frequently accompanies the developmental stage of thrombophlebitis or pulmonary "thromboembolization" or both. He recommended the use of 12.5 per cent plasma to detect this increase in prothrombin. Brambel¹ observed an increased clotting rate when he determined prothrombin time on 12.5 per cent plasma, in various thrombosing conditions. Volkert and Piper⁷ in a study of 6 patients with recurrent pulmonary infarction found an increase of 100 per cent in the variable antithrombin component of the blood. In patients with less pronounced thrombotic conditions, normal values were found. Nielsen⁵ failed to demonstrate variations from the normal antithrombin in 6 patients with established thrombosis.

In a study of various coagulation factors in a series of patients with clinical intravascular thrombosis and embolism we determined the prothrombin by both the Quick and the two-stage method and also the antithrombic activity of the serum of 63 such patients.

This series of patients included the following: 18 with arteriosclerosis obliterans (two of whom subsequently died of postoperative pulmonary embolism), 15 with thrombo-angiitis obliterans, 3 with peripheral arterial embolism, 8 with old thrombophlebitis, 17 with acute thrombophlebitis (3 of whom also had had recent pulmonary embolism) and 2 who had had pulmonary embolism without clinical evidence of thrombophlebitis.

METHODS

The Quick prothrombin test as previously described³ was employed. Standardized thromboplastin prepared in this laboratory was used in 26 cases (Table 1); thromboplastin (The Maltine Company), in 20 other cases. In all instances the activity of individual preparations of thromboplastin was tested by the determination of prothrombin time on plasmas of selected normals using both undiluted plasma and plasma diluted with physiologic salt solution.

Prothrombin was determined by a modification of Herbert's (two-stage) method using commercially available reagents.⁴ Serum antithrombin was determined by a modification of the method of Astrup and Darling using com-

* Received for publication, June 28, 1947.

mercial preparations of thrombin and fibrinogen.⁴ One or more normal plasmas were used as controls each time the determinations of prothrombin and antithrombin were performed.

RESULTS

No clear-cut differences were observed between the various types of thrombosing diseases studied. The patients as a single group were therefore compared with the normal (Tables 1 and 2).

TABLE 1
VALUES OF PROTHROMBIN (QUICK'S METHOD) IN UNDILUTED AND DILUTED PLASMA
IN PERSONS WITH A THROMBOSING TENDENCY

PLASMA CONCENTRATION	CASES	RANGE OF PROTHROMBIN VALUES per cent	AVERAGE PROTHROMBIN VALUE per cent	EXPECTED PROTHROMBIN VALUE per cent
Undiluted	62	50-100	81	100
20 per cent	26	11-25	17	20
12.5 per cent	26	9-14.5	11.6	12.5

TABLE 2
COMPARISON BETWEEN NORMAL PERSONS AND THOSE SHOWING A THROMBOSING TENDENCY,
IN THE VALUES OF PROTHROMBIN (TWO-STAGE METHOD) AND ANTITHROMBIN

	NORMAL	THROMBOSING DISEASES
Total cases, two-stage prothrombin determinations.....	30	39
Range of values.....	78-119%	45-191%
Average.....	100%	99%
Number of cases above 120%.....	0	10
Number of cases below 80%.....	1	8
Total cases, antithrombin determinations..	47	57
Range of values.....	73-119%	65-154%
Average.....	100%	99%
Number of cases above 120%.....	0	8
Number of cases below 80%.....	1	10

Antithrombin and two-stage prothrombin values for a considerable proportion of the thrombosing patients were found to lie outside the observed normal range. These patients showed equal tendencies toward high and low values, so that the average for the group represented a normal value. Prothrombin levels by the Quick method (Table 1) on both diluted and undiluted plasma showed a slightly but definitely subnormal trend. Percentage levels of prothrombin were calculated by interpolation from the curve established for the standard thromboplastin used in this laboratory. When thromboplastin (The Maltine Company) was used in the determination of prothrombin time in 20 cases, a shortened time with the 12.5 per cent plasma was observed in only one instance. Of the

10 cases with prothrombin above 120 per cent of normal (in one case reaching 191 per cent of normal), none showed hyperprothrombinemia as measured by the determination of prothrombin time on diluted plasma. In this study, therefore, the determination of prothrombin time on diluted plasma gave no more information than the determination on undiluted plasma.

COMMENT

The principal justification for presenting these largely negative observations is that there is good reason to suspect some derangement of the coagulation process in many patients who have a tendency toward thrombosis. From a clinical point of view, vascular pathologic changes often seem inadequate as the sole explanation of intravascular thrombosis. Laboratory evidence, such as the coagulation time of heparinized blood *in vitro*, in a general way indicates some abnormality, but no known specific coagulation factor has been conclusively implicated. The variations from normal herein reported also seem best interpreted as indicating some disturbance of the coagulation mechanism but the factors actually studied do not appear to be primarily concerned. With regard to the contention that hyperprothrombinemia may be detectable only in diluted plasma, it may be worth while to call attention to the evidence presented by Deutsch and Gerarde² indicating that fibrinogen concentration may be a limiting factor in the prothrombin times obtained with saline-diluted plasmas. Unusually short prothrombin times with 12.5 per cent plasma thus may represent increases in fibrinogen, a change of frequent occurrence but probably of questionable significance, in thrombosing tendencies.

SUMMARY

In a series of patients with various thrombosing tendencies, levels of anti-thrombin and prothrombin were often outside of observed normal limits. Low and high values, however, were encountered in equal numbers. The dilution of plasma in order to detect variations in prothrombin value (one-stage method) added nothing to the determination on undiluted plasma.

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VARIATIONS IN PROTHROMBIN AND ANTITHROMBIN FOLLOWING THE ADMINISTRATION OF DICUMAROL*

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Many papers have been written which stress the importance of the prothrombin time test as a guide to the administration of Dicumarol. In the determination of the prothrombin time, the one-stage method of Quick or some modification of it has been employed. This method consists in the determination of the clotting time of oxalated plasma at 37.5 C. after the addition of an excess of thromboplastin and a fixed amount of calcium. The time thus obtained is dependent on the conversion time of prothrombin to thrombin as well as on the amount of prothrombin present in the blood.

The two-stage method, originally described by Warner, Brinkhous and Smith,⁶ has not been used clinically after the administration of Dicumarol because it is time-consuming. This method utilizes the biphasic nature of the clotting reaction. In the prothrombin conversion stage, prothrombin is converted to thrombin with an optimal amount of calcium and an excess of thromboplastin. In the clotting stage, the amount of thrombin is measured by the time required for the clotting of a standard fibrinogen solution.

In few instances have tests by these two methods been performed simultaneously. Higher levels of prothrombin with the Quick method have been reported, however, in normal infants,⁴ dogs and rabbits⁷ and in patients with pernicious anemia⁸ and with hepatic disease.⁹ In normal persons the two methods agree very well.

Since the amount of prothrombin in the blood is reduced by Dicumarol therapy, it was decided to determine prothrombin by both the one-stage and the two-stage methods after the administration of Dicumarol. It was further decided to determine the antithrombin content of the blood at the same time.

METHODS FOR THE DETERMINATION OF PROTHROMBIN

One-stage method. The method of Quick as previously described¹ was employed using a standardized thromboplastin.

Two-stage method. A modification of Herbert's method was employed using commercially available reagents.² With this technic, 30 normal persons showed prothrombin levels of from 244 to 378 units per cubic centimeter of plasma, the average being 317 units.

METHOD FOR THE DETERMINATION OF ANTITHROMBIN

A modification of Astrup and Darling's method was employed using commercially available reagents.² The standard for antithrombin was established on 47 normal persons as described. The range of normal variation observed was from 73 per cent to 119 per cent of

* Received for publication, June 28, 1947.

the normal average. One or more normal plasmas were used as controls each time the determinations of prothrombin and antithrombin were performed. It was necessary to use the same normal subjects on numerous occasions. This procedure enabled one to check any possible daily fluctuation in the activity of the reagents used.

RESULTS

In the beginning of this study, plasmas showing different degrees of prothrombin deficiency as measured by the Quick test were selected at random and the amount of prothrombin was determined by the two-stage method. The prothrombin times in seconds indicated by the Quick test were expressed in terms of percentage of normal prothrombin using an average dilution curve of normal plasmas (that is, if a prothrombin time of 35 seconds is obtained on a 20 per cent concentration of normal plasma, then an unknown plasma giving a prothrombin time of 35 seconds may for purposes of comparison be said to contain 20 per cent of normal prothrombin). Percentages of normal prothrombin as measured by the Quick method were then compared with percentages of normal prothrombin

TABLE 1
SIMULTANEOUS ONE-STAGE AND TWO-STAGE PROTHROMBIN VALUES ON PATIENTS RECEIVING DICUMAROL

	SPECIMEN									
	1	2	3	4	5	6	7	8	9	10
Percentage normal prothrombin..... (one-stage method)	22	16	18	35	14	20	14	13	55	20
Per centage normal prothrombin..... (two-stage method)	62	56	46	75	49	56	39	35	76	34

as measured by the two-stage method.* In many instances, results obtained with the two methods were comparable. In many others, marked differences were observed, the Quick test usually indicating the lower level of prothrombin (Table 1).

After repeated determinations on seven different patients, it soon became apparent that the differences in levels or amounts of prothrombin obtained by the two methods were somewhat dependent on the interval of time which had elapsed since administration of the initial dose of Dicumarol and on the total amount of Dicumarol which had been administered; the differences were more marked in some patients than in others.

* In normal plasmas, the percentage of normal prothrombin may be more than 100 as measured by the two-stage method; that is, prothrombin may be in excess of the average normal value. With the Quick test, any increase above the normal prothrombin cannot be demonstrated. With the method of Quick employed, an increase in prothrombin time of only two seconds may represent a decrease to 80 per cent of normal prothrombin. When comparing relative changes in prothrombin activity resulting from Dicumarol therapy, relative differences between the two methods can best be compared when the prothrombin by both methods is less than 80 per cent of normal.

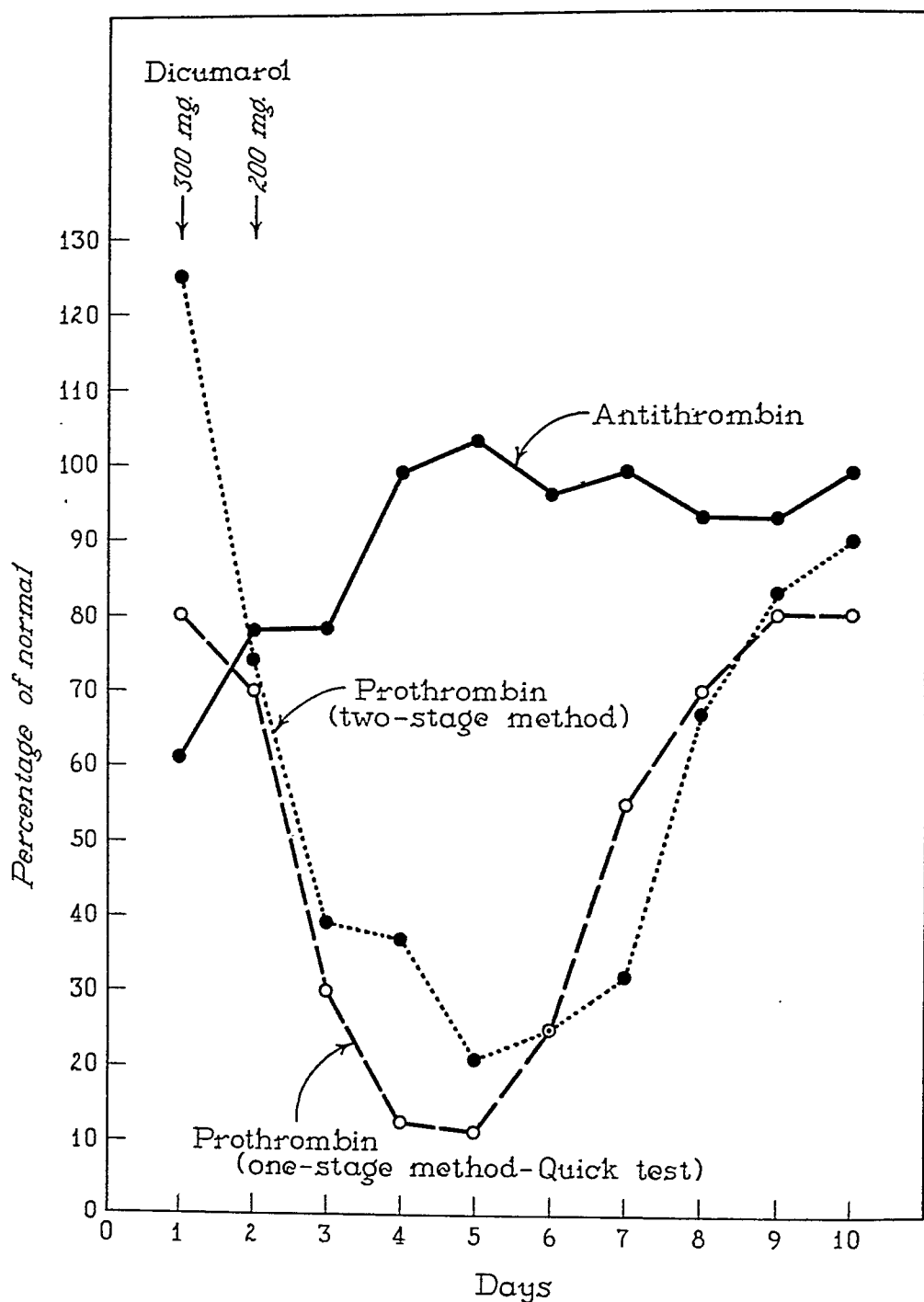


FIG. 1. The effect of Dicumarol on prothrombin (as determined by one-stage and two-stage methods) and antithrombin in a patient showing a relatively transient effect from two initial doses of the drug.

Daily determinations of prothrombin were then performed on eight different persons. The length of time each was studied varied from seven to fifteen days. Results obtained on three patients are shown in Figures 1 to 3.

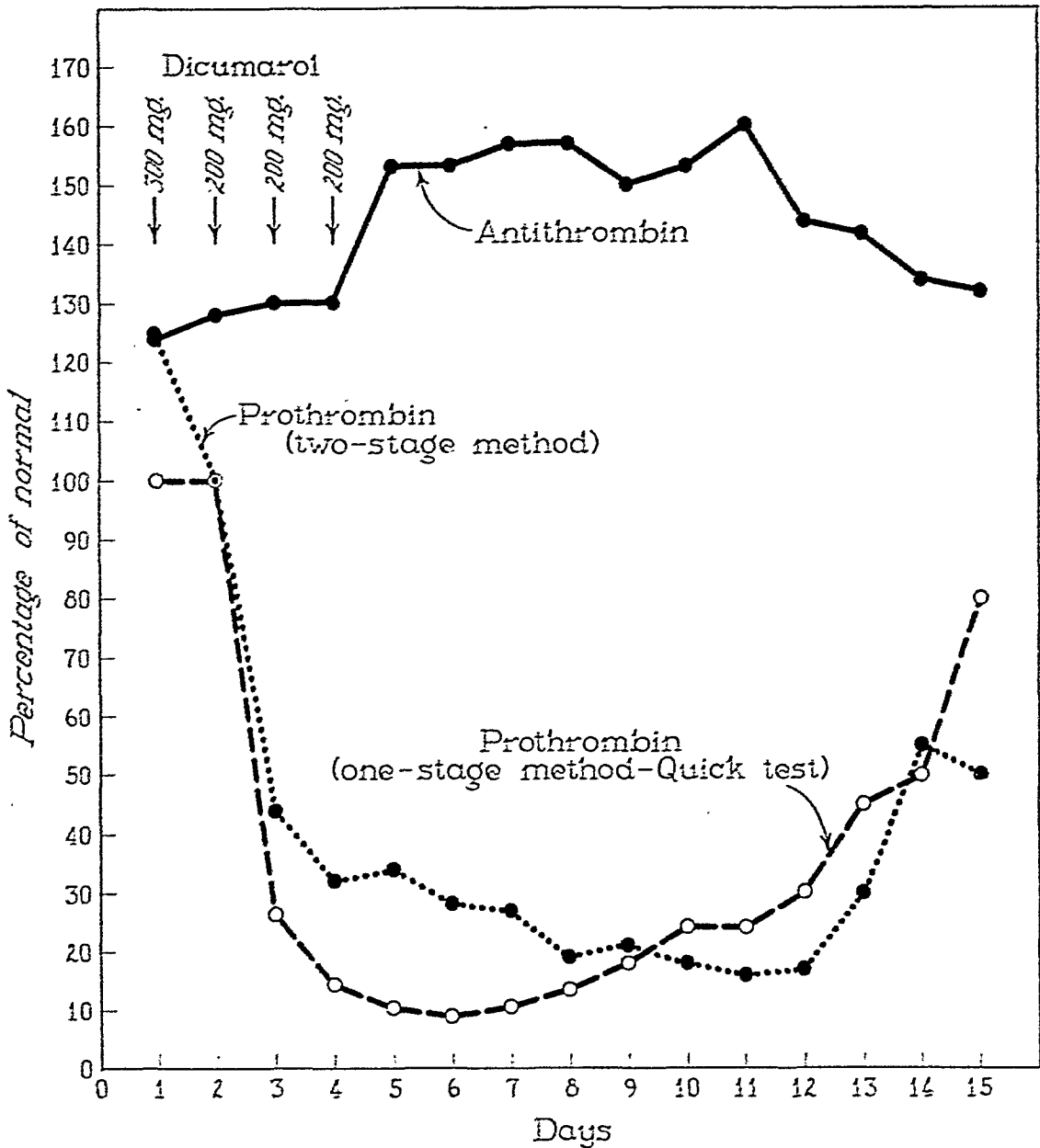


FIG. 2. The effect of Dicumarol on prothrombin (as determined by one-stage and two-stage methods) and antithrombin in a patient showing a relatively prolonged effect of four initial doses of the drug.

In the eight patients who had received Dicumarol, lower levels of prothrombin were indicated by the Quick test than by the two-stage test early in the course of treatment. After administration of Dicumarol was discontinued and its effect diminished there was a tendency for this difference to disappear and finally for the Quick test to indicate slightly higher levels of prothrombin than those obtained with the two-stage test.

Since numerous workers have preferred to use diluted plasma instead of, or in addition to, the undiluted plasma in the one-stage method, prothrombin times were also determined on 50, 20 and 12.5 per cent plasma except in those instances in which the higher dilutions gave unsatisfactory results. As plasmas became

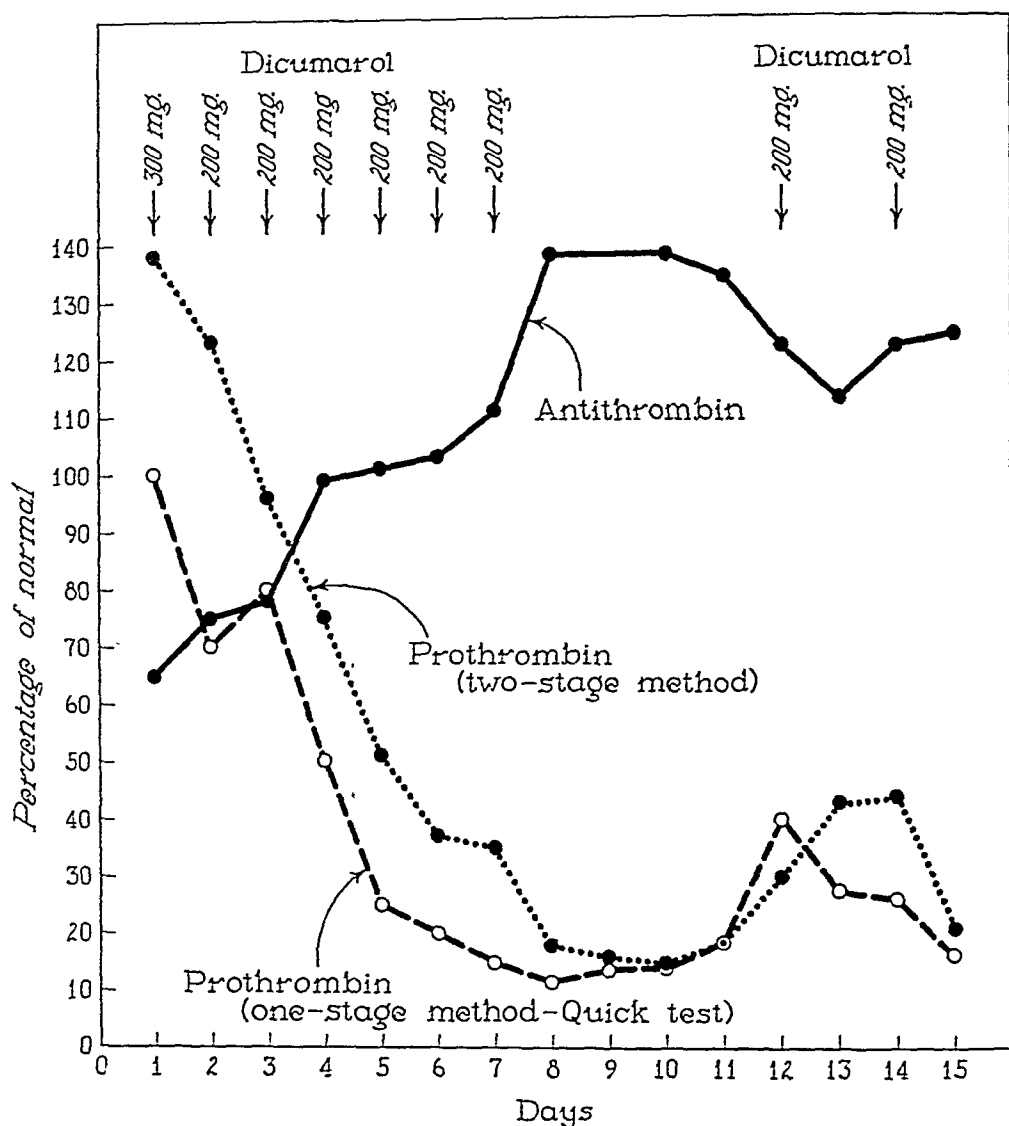


FIG. 3. The effect of Dicumarol on prothrombin (as determined by one-stage and two-stage methods) and antithrombin in a patient who was relatively resistant to the drug.

more deficient in prothrombin (Quick test) the calculated prothrombin percentage on dilutions usually indicated higher levels, although they were still markedly lower than those with the two-stage test. As prothrombin time (Quick test) returned to normal, calculated percentages with diluted plasmas were still higher than those calculated from undiluted plasmas, a fact which made the increase above the two-stage values even greater.

On numerous occasions, elevated antithrombins were observed on the random samples of plasmas first studied. When daily serum antithrombin determinations were performed on the eight selected patients a 40 to 80 per cent increase in antithrombin activity was observed after the administration of Dicumarol. Within certain limits, this increase was rather closely correlated with the decrease in prothrombin.

COMMENT

Instances of higher levels of prothrombin determined by the one-stage method have been explained by the originators of the two-stage method as being due to an increased rate of conversion of prothrombin to thrombin. Quick⁵ has explained this difference in results obtained with the two methods on the basis of incomplete conversion of the prothrombin in the higher dilutions required by the two-stage technic.

In normal plasma after storage, lower levels of prothrombin are indicated by the Quick method.³ With plasma of patients treated with Dicumarol the Quick test also gives lower values than the two-stage procedure. The question must be considered whether the observed increase in antithrombin may affect the assay values for prothrombin. If the lower values obtained with the Quick method are to be attributed to the influence of antithrombin, then the one-stage method must be more affected by antithrombin than the two-stage method. However, when normal plasma was diluted with four volumes of plasma having a value of antithrombin of 200 per cent and of prothrombin of less than 5 per cent, the prothrombin time was that of normal plasma similarly diluted with prothrombin-free plasma. It will also be noted (Figs. 1 to 3) that as the effect of Dicumarol diminished, the prothrombin by the one-stage method equaled or exceeded the two-stage values while antithrombin remained distinctly elevated. With the information available at the present time, the lower levels indicated by the Quick method can best be explained on the basis of a decreased rate of conversion of prothrombin to thrombin. It is not to be expected that results obtained with the one-stage and two-stage methods will necessarily agree although information from either test may be useful. Since ordinarily coagulation occurs when only a small fraction of the total prothrombin has been converted to thrombin, it is evident that the rate of conversion is usually more important than the total amount of prothrombin. Hence the Quick test, in which conversion rate markedly influences the observed prothrombin time, has proved to be the indispensable guide to Dicumarol therapy. A combination of the two-stage and the one-stage procedure yields specific information regarding the rate of conversion.

SUMMARY

By both the two-stage method and the Quick method, a marked decrease in prothrombin level was observed after the administration of Dicumarol.

Early in the course of the Dicumarol therapy, the levels of prothrombin as established by the Quick test were always less than, often less than one-half, those obtained with the two-stage method. When administration of Dicumarol

was discontinued, there was a tendency for this difference to disappear and finally for the one-stage method to give slightly higher values than the two-stage method. This difference in results obtained with the two methods would seem to indicate a decreased rate of conversion of prothrombin to thrombin.

In all instances there was an increase in the antithrombin activity of the serum after the administration of Dicumarol.

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ANTHRAX MENINGITIS.

REPORT OF A CASE OF INTERNAL ANTHRAX WITH RECOVERY*

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Anthrax meningitis has always been considered malignant and we have been unable to find any reports in the literature of its successful treatment. Favorable results, however, have been reported of the use of penicillin in experimental animal anthrax¹ and there are also recent reports of the cure of cutaneous anthrax in man by the use of penicillin.³

A resume of 66 cases of anthrax at the Alexander Smith and Sons Carpet Company² revealed four deaths. One case was complicated by diabetes mellitus, and the other three patients died of anthrax meningitis.

REPORT OF CASE

A white man, aged 57, was employed as a wool picker at the carpet shop for two weeks. He presented himself at the infirmary on September 23, 1946, showing a vesicular lesion on the upper lip, 0.5 cm. in diameter, surrounded by edema, which had been present for twenty-four hours. Anthrax was suspected, the patient was hospitalized at once, and a culture and smear were made from the lesion. Numerous gram-positive bacilli having the morphology of *B. anthracis* were seen and a culture and inoculation of a mouse confirmed the diagnosis of anthrax infection.

First day of hospitalization (September 24). The initial treatment consisted of 100,000 units of penicillin followed by 100 cc. of antianthrax serum (Lederle Laboratories, Inc., New York, N. Y.) both of which were given intramuscularly.

Second day. The temperature rose to 102 F. and the right submaxillary lymph glands became enlarged. Pain and bulging behind the right eye indicated pressure, suggesting cavernous sinus thrombosis. During the night, it was noted that the patient developed some twitching in the arms and face; he complained of pain in the back of the neck. Cultures of blood and pus were positive for *B. anthracis*.

A spinal tap was performed and the fluid appeared clear, the cell count was 0, the sugar, chloride and globulin were all normal. A culture of the cerebrospinal fluid was taken and a guinea pig inoculation was performed. Despite the normal microscopic and chemical findings, meningeal involvement was suspected and 30,000 units of penicillin were injected intrathecally. The patient was given 4 gm. of sulfadiazine initially, followed by 1 gm. every four hours. Culture was made and *B. anthracis* was isolated from the otherwise negative spinal fluid.

* Received for publication, June 23, 1947.

Eight hours later, the temperature rose to 103.8 F. and the patient developed rigidity of the neck, a Kernig's sign, and a Babinski's sign. A second spinal tap at this time revealed very cloudy fluid, a cell count of 3500 cells per cu. mm., \pm plus globulin, negative sugar, 1200 mg. protein, and 760 mg. chloride.



FIG. 1. Photograph of patient taken October 1, 1946, on eighth day of hospitalization.

Twenty thousand units of penicillin were again injected intrathecally, and cultures were again made and reported as positive twenty-four hours later.

Third day. During the night, the temperature began to fall; by 8:00 A.M. it was 102 F. Another spinal tap was performed. The cell count was 328 per cu. mm., sugar 0, globulin 0. Twenty thousand units of penicillin were injected intrathecally and a spinal tap at 7:00 P.M. revealed 42 cells per cu.

mm. Twenty thousand units of penicillin were again injected intrathecally and by midnight the temperature was 100 F. Culture of this fluid was positive for *B. anthracis*.

Fourth day. A spinal fluid examination at 8:00 A.M. revealed a cell count of 22 cells per cu. mm., normal reduction of sugar and negative globulin. Another 20,000 units of penicillin were administered intrathecally. Culture of the spinal fluid was negative.

Fifth to eighth days. The patient's temperature remained within normal limits; his meningeal irritation slowly disappeared and the lesion took on the appearance of a black, gangrenous, crusted area measuring 1.5 cm. in diameter. The edema surrounding the area disappeared and the enlarged glands in the neck also subsided.

TABLE 1

SUMMARY OF MEDICATION GIVEN TO PATIENT IN TREATMENT OF ANTHRAX INFECTION OF LIP AND MENINGES

1. Total of 500 cc. of antianthrax serum intramuscularly: 100 cc. given September 23 1946, and 50 cc. given every eight hours thereafter for 8 doses, up to September 27 1946.
2. Total of 110,000 units of penicillin intrathecally: 30,000 units given initially followed by 20,000 units every twelve hours for 5 doses.
3. Total of 4,400,000 units of penicillin intramuscularly: 100,000 units given every three hours from September 23 to September 27, 1946, and 50,000 units given every three hours from September 27 to September 30, 1946.
4. Continuous 1:4000 wet penicillin dressing to lesion.
5. Total of 28 gm. sulfadiazine: Four gm. given initially followed by 1 gm. every four hours from September 24 to September 28, 1946.

Ninth day. The patient developed the following symptoms of serum sickness: pruritus, urticaria, back pain and a mild rise in temperature. All of these symptoms disappeared within the next two days.

Fifteenth day. The hard crusted lesion dropped off the upper lip leaving no visible scar.

Twenty-eighth day. The patient was able to report back to work.

SUMMARY AND CONCLUSIONS

From a review of the literature and from the cases of infection at a carpet shop, one is impressed with the following facts:

1. While anthrax infection is rare, its occurrence among wool workers is sufficiently frequent that one should always be suspicious of cutaneous lesions in such workers.

2. The incidence of meningeal involvement in anthrax infection is about 5 per cent. In the treatment of anthrax one should be constantly on the alert for evidence of meningeal involvement.

3. Treatment for meningeal anthrax infection should be instituted immediately upon clinical evidence of meningeal irritation. In the case reported here, the

appearance of the first spinal fluid taken was entirely negative but mouse inoculation was positive and culture was reported positive twenty-four hours after the treatment was started. There was excellent response to the medication which consisted of penicillin intramuscularly and intrathecally, and locally to a skin lesion, antianthrax serum intramuscularly and sulfadiazine by mouth.

4. Owing to the rapid development of meningeal involvement in anthrax infection, it appears that antianthrax serum alone is ineffective, but in this case the penicillin may have inhibited the growth of the organisms until the antiserum had sufficient time to act. Inasmuch as previous cases of anthrax meningitis treated without penicillin all terminated fatally, one may speculate on a synergistic action between the antianthrax serum and penicillin in this case.

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DEATHS FOLLOWING USE OF ABORTIFACIENT PASTE.

REPORT OF TWO CASES*

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The use of an abortifacient paste to supplant operative intervention was initiated by Heiser of Berlin and advocated by Leunbach of Copenhagen in 1931. The first favorable articles in the German medical press, however, were quickly followed by numerous reports emphasizing serious effects which followed the use of this paste. Several editorials in the Journal of the American Medical Association³ warned against its use. Results of experiments conducted to demonstrate the deleterious effect of such preparations were published by Otto⁴ and Brack¹ in 1932. The pastes have appeared under the trade names of "interruptin" (Heiser), "provocol" (Leunbach) and "ultra jel" (in the United States). They consist essentially of soaps made from castor oil, cocoa butter or other vegetable oils, combined with iodine ($1\frac{1}{2}$ to 4 per cent of iodine), thymol and tincture of benzoin. In 1944, Weilerstein⁷ described three sudden deaths in this country caused by the use of this abortifacient.

Weilerstein demonstrated some of the effects of the pastes in dogs and Straus and De Nosaquo⁶ in rabbits and rats. These workers stated that necrosis of the uterine wall due to caustic action was sometimes delayed and difficult to differentiate from a perforating injury due to a mechanical procedure. At autopsy a careful investigation may be necessary to determine the rôle played by the paste.

The following two case reports are from the records of the Cuyahoga County Coroner's Office. In the first case, death occurred about two and one-half months after the injection of the paste which had resulted in incomplete abortion and extensive necrosis of the uterine wall followed by general peritonitis. In the second case, sudden death occurred a few hours after an attempted abortion by use of a paste. The patient had presented symptoms of pulmonary embolism; emboli were found which were composed of fatlike particles and particles of damaged placenta.

REPORT OF CASES

Case 1

Clinical data. A 20 year old white woman died with bilateral parametrial abscesses and symptoms of general peritonitis. The parametrial abscesses had been drained and the patient had been subjected to intensive treatment with sulfa drugs and penicillin. The remnants of a placenta and an embryo of about two months' gestation had been removed by instruments approximately two months after the attempted abortion.

Gross findings. The body was jaundiced and somewhat emaciated. Foul smelling, cloudy, yellowish green fluid exuded from the vagina. On opening the abdomen, there was

* Received for publication, April 5, 1947.

a yellowish green discoloration of the peritoneum, multiple recent adhesions of the grayish red coils of the small intestine to the posterior wall of the fundus of the uterus, and of the sigmoid to the left lateral wall of the uterus and to the parametrium, obliterating the cul-de-sac. Both ovaries and tubes were included in the adhesions by the inflammatory process. The uterine body measured 6 cm. in length, 7 cm. in the frontal diameter and 3.5 cm. in the sagittal diameter. There was extensive necrosis of the uterine wall. On cross section, the necrosis was most marked in the lower portions above the inner cervical opening. The cervix measured 2 cm. in length, was grayish red, thickened and edematous. The rugae of the grayish red vagina were well preserved. In the parametrial folds at either side of the uterus were cavities measuring about 3 x 2 cm. filled with foul smelling, cloudy, yellowish brown fluid. These cavities communicated posteriorly with the roof of the vagina through fistulous openings. Veins at both sides presented multiple thrombi. Hypostatic pneumonia with recent pleural adhesions, myocardial degeneration and lymphadenitis of the mesenteric and retroperitoneal lymph nodes were also present.

Microscopic findings. Sections of the uterine wall presented a picture of extensive coagulative necrosis of the wall itself with destruction of the uterine mucosa; no remnants of placental tissue were seen. Masses of mononuclear and polymorphonuclear white blood cells in a network of fibrin filled the stroma of the uterine wall and the parametrium. Sections of the parametrium showed infiltration with round cells. Some thrombosed dilated veins were filled with clumps of amorphous material. Frozen sections of the uterine wall, stained with sudan III, revealed the red particles in the spaces between the bundles of muscle fibers (Spalten infiltration of Brack). The layers of decidual cells and some phagocytes enclosed the fatlike granules.

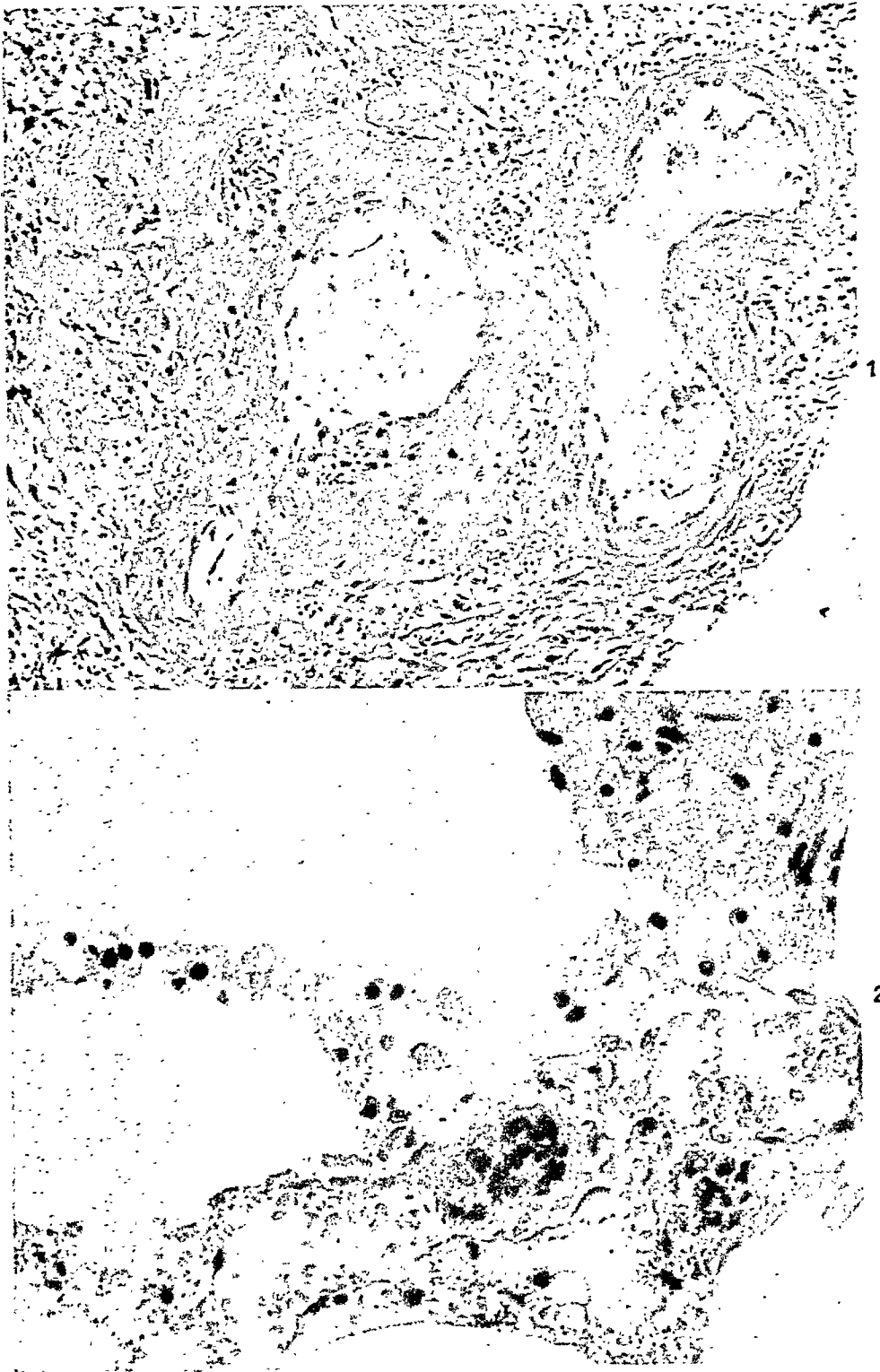
Case 2

Clinical data. A 30 year old colored woman died suddenly in a doctor's office after suffering from lower abdominal pains and respiratory distress. Her uterus was found to be enlarged and corresponded to a pregnancy of from four to four and one-half months.

Gross findings. The lungs were greatly expanded and together weighed 1150 gm. The external surfaces and cross sections of the lungs were reddish pink with dark red areas. Cut surfaces remained on the level of the sections and a pinkish foam exuded. Pinkish frothy fluid was found in the trachea and bronchi. The heart was relatively small but the right ventricle was dilated. There was some bloody fluid in the pericardial sac. The rugae of the vagina were flattened. There were two small superficial lacerations on each side of the cervix. The cervical canal was dilated to 15 x 18 mm. and was filled with a grayish brown, soft, jelly-like material. The same kind of material was seen along the inside of the posterior wall of the lower uterine segment and was more pronounced on the left side than on the right side. The material felt soapy, was alkaline in reaction and had a strong odor of iodine. An enlarged uterus extended half way between the umbilical line and the symphysis; the veins in the parametrium were enlarged and filled with clotted material. No signs of perimetritis were noted. Enclosed in the uterus was an intact embryonic sac adherent to the superior and anterior wall. The inferior pole of the embryonic sac showed a grayish discoloration and was covered with a layer of soft material. The embryonic membranes in this area were edematous and loosened and also showed a grayish discoloration. The embryonic sac contained a male embryo 17 cm. in length and about 150 cc. of yellowish cloudy fluid which had a soapy odor. The embryo showed no gross lesions. The placenta could be stripped off easily, and when removed, measured 11 cm. in diameter. The perimetrium of the uterus was smooth. The uterine wall was hypertrophic corresponding with a pregnancy of four and one-half months' gestation. The veins in the left parametrium

FIG. 1. (Case 2). Section from left parametrium showing necrosis and decidua-like reaction of and about walls of blood vessels.

FIG. 2. (Case 2). Section of lung showing dilated capillaries, containing a multinucleated giant cell.



were markedly dilated and filled with clotted blood. There were no marked changes in the right parametrium. The right ovary contained a lutein body, 11 mm. in diameter.

Microscopic findings. Eight sections of *lung* showed the alveoli to be irregularly widened and filled with a plasma-like material. The alveolar capillaries were irregularly widened, some greatly distended with blood while others were narrowed and emptied. Frequently, clumps of polymorphonuclear giant cells and particles of syncytium were seen dilating and obstructing the pulmonary capillaries (Fig. 2). Frozen sections stained with sudan III (12 sections) demonstrated that the small branches of the pulmonary artery and networks of capillaries of the alveoli were filled with a purplish red stained material. There was hypertrophy of the musculature of the *uterus*. The mucosa was markedly flattened and was occupied by decidual cells. Frozen sections of the uterine wall taken from the discolored area and stained with sudan III showed red stained particles in the decidua and in the spaces between the bundles of uterine muscle fibers. This corresponded to those previously described by Brack.¹ The *chorionic villi* were surrounded by a ring of syncytium and enclosed numerous Langhans cells. Some of the villi showed coagulative necrosis. The same type of necrosis was seen in the adjacent layers of the cells of the decidua and of the walls of the enclosed vessels and capillaries. Groups of mononuclear and polymorphonuclear white blood cells surrounded the necrotic area. Cross sections taken from the left *parametrium* showed dilatation of the veins which were filled with a clotted material resembling the orange colored granular material described by Straus.⁶ Occasionally, a chorionic villus-like form was seen. The walls of some of the parametrial vessels showed coagulative necrosis (Fig. 1). Sections from the *pituitary gland* and from the *breasts* presented evidences of pregnancy.

COMMENTS

The use of a fat stain (sudan III) facilitated the findings of particles of the paste in the affected uterine wall as well as in the lungs. The stained particles appeared purplish red and differed from the usual orange-red which ordinarily demonstrates the presence of fat. Controls of uterine tissue from both pregnant and nonpregnant individuals obtained at autopsy and treated in an identical manner failed to show such stained particles.

The microscopic picture of the lungs of the patient in Case 2 differed markedly from that described by Steiner and Lushbaugh,⁵ in their cases of pulmonary embolism by amniotic fluids which occurred during and following delivery.

The appearance of syncytial giant cells derived from damaged chorionic villi during delivery, and more frequently, after puerperal eclampsia, or after manual or instrumental removal of the placenta and other manipulations, was described fully by Celen.² However, the presence of a markedly increased number of such forms in the pulmonary capillaries causing dilatation and obstruction in such an early and grossly intact pregnancy as in Case 2 seems unusual.

SUMMARY

The autopsy findings are described in two patients in whom death followed the injection of an abortifacient paste. In one patient, death occurred two and one-half months after the attempted abortion. Necrosis of the uterine wall, parametrial abscesses and generalized peritonitis were found.

The second patient died suddenly of pulmonary embolism within a few hours after an attempt to cause abortion by intra-uterine injection of the paste. The emboli were composed of fatty material and particles from the damaged chorionic

villi. There was necrosis of some of the villi and vessels of the uterine wall but no apparent changes in the embryo or the embryonic sac. The presence of a pasty material was demonstrated by the technic used routinely for the demonstration of fat but the staining reaction was different from that of fat.

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EDITORIALS

THE PHASE MICROSCOPE

The phase microscope, which was demonstrated for the first time in the United States in 1944 at the Cleveland Meeting of the American Association for the Advancement of Science, is now used in various general research institutes. It is also employed in the morphologic field of cancer research and eventually may be a useful apparatus in a histologic laboratory. Bennett (Bennett, A. H.: Phase microscopy, its development and utility. *The Scientific Monthly*, **63**: 191-193, 1946) of the American Optical Company, and Bennett, Jupnik and co-workers (Bennett, A. H., Jupnik, H., Osterberg, H., Richards, O. W.: Phase microscopy. *Transactions of the American Microscopical Society*, **45**: 99, 1946) have contributed much to the perfection and use of the phase microscope. The following paragraph is quoted from their articles. Von Albertini has used the phase microscope rather extensively and his more recent studies also will be reviewed.

There are many microscopic materials which are transparent, or nearly so, whose details differ from their background in either refractive index or actual thickness, resulting in a difference in light path. The purpose of phase microscopy is to render such details visible with sufficient resolving power for their interpretation. Phase microscopy is advantageous for viewing structures, which have small differences in what is called the optical path. The optical path expressed in wave lengths " λ " is equal to nt/λ , where " t " is the thickness and " n " is the index of the refraction of the structure. The microscopic field may show small differences in light transmission only, which can be amplified in the image by the method of phase microscopy. In many cases unwanted details can be suppressed and others emphasized. These details may involve amplitude (transmission) or phase (optical path) differences, or both. Corresponding to a given phase and transmission difference in the specimen, suitable phase and absorption distributions are introduced elsewhere in the optical system in order to alter the contrast in the image. This method of varying contrast in the image by utilizing absorption and optical path differences in the optical system is called "phase microscopy", or "phase difference", or "contrast microscopy".

Von Albertini (von Albertini, A.: Vergleichende histologische Geschwulstuntersuchungen mit dem Phasenkontrastverfahren. *Schweiz. Ztschr. Path. u. Bakt.*, **10**: 4-29, 1947) studied a number of malignant tumors by means of phase microscopy. The advantage of this method lies in the possibility of examining fresh tissue, unfixed and unstained. Teased preparations were used and also specimens obtained from washings of tumor tissues. By the use of phase microscopy it was shown that the tumor cells are not coherent and that nuclei and cytoplasm are not firmly bound together, but are easily separated. This phenomenon is called "inconstancy of the cytoplasm". It cannot be observed in fixed tissues and is demonstrable only by the use of phase micro-

scopy. This inconstancy of the cytoplasm of tumor cells is indirectly proportional to the degree of the maturity of tumor cells.

In stained preparations, examined by means of the standard microscope, attention is usually paid only to nuclear changes. In regard to the cytoplasm mention is made only of the presence of granules or droplets, and of the presence or absence of mucus. Changes within the cytoplasm can be studied better by phase microscopy.

In the study of squamous cell carcinomas, phase microscopy is of special advantage. Stress is laid upon the presence of tonofibrils, desmosomes and intracellular bridges, which are present in and between normal squamous cells. Because of these structures, squamous cells are coherent. In instances of squamous cell carcinoma, however, these cohesions are lost early and the cells are either only loosely attached to one another or are free. Also the nuclei are not bound to the cytoplasm, but appear "naked". The absence of tonofibrils, desmosomes and intracellular bridges should, therefore, raise suspicion of early changes in the direction of malignancy.

From the study of various tumors by means of both routinely stained sections and phase microscopy, von Albertini believes that in the determination of the degree of malignancy not so much stress should be laid upon the degree of differentiation of the cells, but rather upon a determination of how much dedifferentiation of the individual tumor cells is present. Dedifferentiation is used in the sense of lessened maturity of the cells and is regarded as being identical with cancerization. Characteristics of dedifferentiation, as observed by phase microscopy, are "naked" nuclei surrounded by finely granular masses of cytoplasm. It is also noted that highly malignant tumors do not consist of firmly packed tissue, but rather of a thick liquid suspension of nuclei in dissolute masses of cytoplasm.

Chicago

OTTO SAPHIR

ON THE TECHNIC OF PUBLIC SPEAKING

It is an old aphorism that a good speaker can make a dry subject interesting and a poor speaker can make the most interesting subject thoroughly boring. It is, therefore, apropos to consider some of the attributes of a good speaker.

Considerations for good speech require that enunciation should be clear, words should not be mouthed, sentences should be short, sharp and pointed. The lower jaw should be moved; the facial muscles should register the spirit of the remarks. Some of the old schools of oratory laid stress upon organized gestures, which resulted in an artificial, stilted type of presentation. Gestures, when used, should be natural and not seem to have been prefabricated. The whole body should take part in this naturalness. Personal peculiarities seldom add to an address and are usually tiring to an audience. Some speakers walk up and down the platform, others cultivate mannerisms much like an advertising agency which repeats and repeats a particular slogan to call attention to a product. In most medical presentations the emphasis should be on the message, and not on the

individual delivering it. Doctors who constantly call attention to themselves are usually stigmatized sooner or later.

The voice should rise and fall with the matter of the text. Monotones are used only for special effects. It is usually better to speak just loud enough and not to shout or raise the voice unduly. To speak too softly is to deprive a part of the audience of what they came to hear. The quantity of sound can be regulated by properly focusing the voice, not in the throat or behind the teeth, but just in front of the lips. The voice can be thrown out, so to speak, over the heads of the audience in an imaginary arc, the other end of which reaches just behind the last person in the room. The bones of the head and the air in the sinuses can be made to vibrate, thus making overtones, increasing carrying power and making the quality of the voice more pleasant. Focusing the voice also avoids harshness and unnecessary strain on the muscles of the throat and larynx.

In English, the consonants are more important than the vowels. They should be emphasized when accentuation is wanted, leaving the vowels to take care of themselves. Thus, if a young man tells his beloved, "I love you", and accents the "I" in love, she is more likely to believe him.

It is well to single out in the audience the one who looks the most inattentive and bored and speak directly to this individual. The rest will probably be interested.

Twenty minutes is about as long a time as an audience will listen to a serious discussion. If the address is to be longer, a story should be told with a point apropos of the subject. A slight pause before the story adds interest. Then ten minutes more of serious discussion can be given and another story told.

Many speakers attempt to cover too much ground in the time allotted. It is better to pick out one or two, or at the most, three points and drive them home. Audiences usually like a narrative style better than a highly formal one. Add personal experiences from time to time about Mrs. A or Mrs. B.

The several points to be emphasized should be well organized. It is not good speaking to repeat constantly, retrace or reiterate, except for special purpose of emphasis. Hemming and hawing are anathema. Audiences enjoy being taken into the speaker's confidence, and this can be done by acting and by tone of voice. Talking "down" to audiences is seldom effective. If the speaker knows that the audience needs basic instruction, it can be done without seeming to do so; nor must one talk "over" his audience. A good speaker never assumes that his listeners know as much as he, for if they did, they would not bother to constitute themselves an audience.

The two chief purposes of addresses are to teach and to exhort; most speeches combine these two purposes. The special difficulty in speaking on health matters lies in the use of technical language. People do not come to health meetings to be taught a language, but to learn facts. In truth, if one understands a subject well, he should be able to tell it to a seven year old. The trick is never to use a technical word without defining it. Sometimes a whole sentence or even a paragraph in the vernacular should be used instead of one technical word.

Finally, a speaker should stop on time, because it is selfish and discourteous to encroach on the time of the next speaker. It is much better to conclude the talk and leave the audience wishing that the speaker would continue, rather than to exceed one's allotted time while the audience wonders, "Oh, when will he stop talking?"

The whole matter warrants considerable effort. It matters little how interested and eager the audience; the speaker must be able to transmit his message.

Philadelphia

STANLEY P. REIMANN

BOOK REVIEWS

The X-ray Treatment of Accessible Cancer. By D. WALDRON SMITHERS, M.D., D.M.R., Director of Radiotherapy Departments, The Royal Cancer Hospital (Free) and the Royal Free Hospital; Radiotherapist Brompton Hospital for Diseases of the Chest; Associate Physician for Radiotherapy, West End Hospital for Nervous Diseases. 147 pp., 101 figs. \$8.50. Baltimore: The Williams and Wilkins Company, 1946.

This is a report of experience with "short-distance low-voltage" or "contact" x-ray therapy of accessible cancer extending over ten years. Though meant primarily for the radiotherapist, the book contains a great deal of valuable information for the pathologist, especially if he is connected with a tumor clinic. Chapters 3 and 4 deal with classification, keeping of records, changes in tumors due to irradiation, problems of biopsy, and with a new field of cytologic investigation: cell counts following irradiation. The final seven chapters take up the various forms of accessible cancer: skin, lip, nose, ear, eyelids, cornea, external genitalia, mouth, cervix, uteri and lymph node metastases. Only gross characteristics of the tumors are presented, as seen by the radiologist. The presentation is clear and brief, but as complete as the subject requires. The illustrations are of high quality, including those in color, as are also the paper and the general appearance of the volume. A useful glossary of clearly worded definitions is appended.

The pathologist plays an increasingly important part in the growing movement against cancer. This book may help him to take better advantage of the opportunities which his strategic position offers.

Chicago

I. DAVIDSOHN

The Modern Attack on Tuberculosis. Revised Edition. By HENRY D. CHADWICK, M.D., Superintendent of Westfield State Sanatorium (1909-1929); Tuberculosis Controller of the City of Detroit (1933); Commissioner of Public Health of the Commonwealth of Massachusetts (1933-1938); Medical Director, Middlesex Tuberculosis Sanatorium (1938-1941); and ALTON S. POPE, M.D., Chief, Bureau of Communicable Disease, Department of Health, Chicago (1926-1929); Deputy Commissioner of Public Health and Director of Division of Tuberculosis, Commonwealth of Massachusetts. 134 pp., 5 figs., 4 tables. \$1.00. New York: The Commonwealth Fund, 1946.

This revised edition brings up-to-date the technic of tuberculosis case-finding and control. The unwillingness of the public and, to some extent, of the medical profession to regard the disease as communicable has retarded its control. In many states from 15 to 20 per cent of cases are reported after death. The authors stress the fact that tuberculosis is becoming a disease of older men. The chapter on diagnostic procedure deals with the development of x-ray equipment and discusses the use of the photofluorograph with the photoelectric timer, which is now in general use in mass surveys. Bronchoscopy should be resorted to more frequently to clear up certain doubtful cases. They do not regard B.C.G. immunization of the general population in the United States as a practical procedure. Adequate hospital and sanatorium beds are necessary for the control of the disease. There are about 98,000 beds now available, which represents an approximate ratio of 1.7 beds to one death per year, the beds being distributed unevenly throughout the country.

In the chapter on case-finding, the point is made that the most productive group for mass examination consists of the family contacts of sputum-positive patients, and of contacts of patients who have died of this disease. One state has reported an incidence of 8.0 per cent among contacts examined. The weakest link in the system of examination of contacts is in securing consent for examination of older members of the family, especially of the male members, among whom the incidence of tuberculosis is highest. The importance is stressed of having physicians take routine x-ray films of all patients, as well as of food-handlers, industrial employees, medical students, nurses, school teachers, school children and patients in mental and general hospitals. Mention is also made of the military inductees

who were rejected for service because of x-ray findings of significant tuberculosis. This group averaged 1.15 per cent for the country at large and could be followed to advantage. Any case-finding program must have the support of the medical profession in order to insure its success. In a campaign to eradicate tuberculosis, information should be obtained on the number of cases of tuberculosis in the community; the special racial, social and economic hazards; the facilities for treatment and segregation; and the available personnel, equipment and funds.

The importance of maintaining a tuberculosis register is emphasized. Voluntary agencies play an important rôle in a community campaign.

In the closing chapter the authors forecast that if the mortality rate from tuberculosis in the United States continues to decline as it has in the last two decades, we may see the death knell of the tubercle bacillus in the year 2000; and, if new developments in treatment come to our aid, it may arrive earlier.

Eloise, Michigan

DAVID LITTLEJOHN

Health Insurance in the United States. By NATHAN SINAI, Dr.P.H., ODIN W. ANDERSON and MELVIN L. DOLLAR. 115 pp., 2 tables. \$1.50. New York: The Commonwealth Fund, 1946.

This monograph is one of a series of studies made under the auspices of a Committee of the New York Academy of Medicine.

The authors have attempted to present the historic data which relate to the development of health insurance in the United States and its present status. They have discussed the attitudes of the various interested groups, including the professional, governmental and lay groups in relation to the whole problem of health insurance. Most attention is given to a review of the various plans of voluntary insurance including an outline of some of the problems involved and the general characteristics of each of these plans. The rapid growth of the movement in the United States from 1933 to 1946 is outlined. The authors point out that, at present, most of the voluntary plans now provide only for such services as hospitalization or surgical and obstetric care, and that few plans make any provision for any type of general medical care, except where hospitalization is necessary.

The authors have rendered a service to all groups and individuals interested in the problem of furnishing adequate medical care to the large body of persons, who for some reason may be unable to finance adequate medical or surgical care when the necessity arises.

DAVID LITTLEJOHN

The Challenge of Polio. By ROLAND H. BERG. 208 pp. \$2.50. New York: The Dial Press, 1946.

Mr. Berg has presented in a clear and factual way the efforts which have been put forth to meet the challenge of securing data necessary to control poliomyelitis. He has outlined much of the research work which has already been done, pointing out the failures as well as the successes, and has emphasized the need for continued research until such time as we know the cause, portals of entry and sources of infection, and develop adequate methods of prevention and treatment of this disease.

The author's discussion of the status of the Sister Kenny concept of the disease and the application of this method of treatment clarifies much of the misunderstanding which has arisen regarding this phase of the polio situation.

DAVID LITTLEJOHN

A History of The American Medical Association, 1847 to 1947. By MORRIS FISHBEIN, M.D., with the biographies of the presidents of the Association by WALTER L. BIERRING, M.D., and with histories of the publications, councils, bureaus and other official bodies. 1226 pp. 176 illus. \$10.00. Philadelphia and London: W. B. Saunders Company, 1947.

This volume serves to point out the aims, ideals and accomplishments of the American Medical Association. From its pages, the reader will readily perceive how American Medi-

cine has become great, and he will, at the same time, acquire some stimulation from the records of the leaders of American Medicine.

Compilation of the history was begun in 1929 and was not completed until 1947. The editing and principal authorship of the history was the work of Morris Fishbein. The biographies of the presidents, written by Walter L. Bierring, president of the Association in 1934, occupies 260 pages and is the most interesting portion of the book. Dr. Nathan Smith Davis, III, wrote the biographic sketch of his grandfather, Dr. Nathan Smith Davis, who was the founder of the Association and the first editor of its Journal. A number of other authors and editors also contributed historic accounts of special activities of the organization.

Pathologists may be interested to note that The Section on Pathology was organized in 1900; that the Archives of Pathology was established in 1926 with Dr. Ludvig Hektoen as its editor; that the Council on Medical Education and Hospitals established essentials for approval of schools for clinical laboratory technicians in 1936, and that in 1946 the Journal listed 268 approved schools.

The history of the American Medical Association is essentially the history of American Medicine. In compiling the 1226 pages of the record of the 100 years of the Association, the editor has fashioned a chronicle of medicine that must be placed beside Garrison's monumental History of Medicine. Truthfully, it may be said that Dr. Fishbein, in his long service on behalf of the American Medical Association, has done fully as much as any other American physician during the past 100 years to help advance American Medicine to the world leadership it now enjoys.

Atlas of Histopathology of the Skin. By G. H. PERCIVAL, M.D., Ph.D., D.P.H., Grant Professor of Dermatology, University of Edinburgh; A. MURRAY DRENNAN, M.D., Professor of Pathology, University of Edinburgh; and T. C. DODDS, F.I.M.L.T., Laboratory Supervisor, Department of Pathology, University of Edinburgh. 494 pp., 376 color photomicrographs. \$16.00. Baltimore: The Williams and Wilkins Company, 1947.

This volume is intended "primarily for post-graduate students and especially those interested in the special study of dermatology". It contains 376 figures, nearly all of which are photomicrographs in color, and most of which are expertly reproduced by the Finlay process. Each of the various sections is introduced by a short descriptive preface. Photographs of microscopic sections of representative lesions are then presented, each with a brief descriptive legend. The sections are arranged according to a clinical classification in which the lesions are grouped with respect to the characteristic primary cutaneous changes rather than according to etiology. The first section is devoted to normal histology of the skin and includes 21 figures. Subsequent sections portray the microscopic changes in the commoner skin diseases. Almost no mention is made of lesions caused by fungi. One is surprised to read that lupus erythematosus still "is generally regarded as having a definite connection with tuberculosis".

The table of contents lists 25 titles, but the various titles do not have equal value. For example, one title, "Rosacea and Rhinophyma", is followed by one-half page of text and one illustration, and another title "Granuloma Pyogenicum" is followed by six lines of text and one illustration. Under the title, "The Naevi", however, there follows a section of 110 pages and 87 figures. A naevus is defined as "a circumscribed hyperplasia of tumor formation which has its origin in aberrant embryological cutaneous structures or rests". Seventeen varieties of naevi are listed and illustrated, including those derived from the epidermis, from the epidermal appendages, from collagen and from vaso-formative cells. This usage of the term is, of course, at variance with that generally adopted by American pathologists who use the term in a restricted sense to indicate a cutaneous neoplasm of neuroepithelial origin. In the section on "Tumours", the authors loosely speak of "tumours such as molluscum contagiosum, the contagious verrucae, and certain of the naevi", and include contagious verrucae, verruca plana, verruca vulgaris, verruca plantaris and condyloma acuminatum.

The atlas is attractive and well printed. For the student of clinical dermatology the book has considerable merit, but as a ready reference for the general pathologist, it falls short of the mark. It is to be hoped that in subsequent editions the need of the pathologist for a more complete and adequate atlas on histopathology may be met, and that stricter attention will be given to terminology. It would seem that the subject of microscopic pathology ought to be treated primarily from the pathologist's point of view rather than from the clinician's. Nevertheless, the authors deserve commendation for the preparation of this atlas since the volume contains numerous illustrations of teaching value. At the present time, this is probably the best atlas on histopathology of the skin in the English language.

Gastritis. By RUDOLPH SCHINDLER, M.D., Clinical Professor of Internal Medicine (Gastroenterology), College of Medical Evangelists, Los Angeles, California. 462 pp., 96 figs., 19 tables. \$8.75. New York: Grune and Stratton, Inc., 1947.

This volume contains a great deal of valuable data but unfortunately much of it is hidden or difficult to find. The fault is primarily in poor organization. The paragraphs are unusually long and discursive. The textual material could be greatly reduced, perhaps by one-half, with considerable advantage. For example, nine and one-half pages are devoted to a definition of gastritis and five of the pages to a definition of inflammation. Additional subheadings would be helpful and the index could be improved.

The author frequently speaks of "tumor-forming gastritis". He defines this term to indicate "only that hyperplasia or proliferation of such degree is present as to produce the picture of a tumor either at x-ray examination, at gastroscopy or at surgical operation". But actually no neoplasm is present in "tumor-forming gastritis".

Among the interesting statements found are the following:

"Taking of routine biopsies with the flexible gastroscope is not yet feasible. Biopsy at laparotomy is preferable."

"Frozen section (of gastric tissue) should not be attempted because the stomach is not a suitable tissue for frozen section."

"In pernicious anemia and in atrophic gastritis, tumors develop more frequently than in other people. It seems that patients with atrophic and atrophic-hyperplastic gastritis have a three times greater chance of developing a gastric carcinoma than the average adult of the same age group."

There are 96 illustrations, almost all of which are large photomicrographs of gastric lesions. These are well reproduced and should prove of interest to pathologists.

NEWS AND NOTICES

COLLEGE OF AMERICAN PATHOLOGISTS

The first general meeting of the College of American Pathologists will be held at the John B. Murphy Memorial Auditorium, 50 East Erie St., Chicago on Monday, October 27, 1947, beginning at 3:00 p. m. Following dinner, an evening convocation will be held, beginning at 8:30 p. m., at which the College will formally induct all members and fellows.

REVISTA CUBANA DE LABORATORIO CLINICO

We wish to extend congratulations to the Sociedad Cubana de Medicos Laboratoristas Clinicos on the publication of its official journal, *Revista Cubana de Laboratorio Clinico*. The new Journal will be published quarterly.

THE AMERICAN SOCIETY FOR THE STUDY OF ARTERIOSCLEROSIS

The organizational meeting of the American Society for the Study of Arteriosclerosis was held in Atlantic City, June 9. The officers of the Society are: Dr. W. C. Hueper, New York, president; Dr. William B. Kountz, St. Louis, vice president; Dr. O. J. Pollak, Wilmington, Delaware, secretary-treasurer.

MICROFILM SERVICE OF ARMY MEDICAL LIBRARY

Through the microfilm service of the Army Medical Library, physicians, librarians and professional workers may obtain facsimile replicas of the great bulk of existing medical literature on 35 millimeter film, according to Colonel J. H. McNinch, Commandant of the Army Medical Library. Articles in medical periodicals are duplicated on microfilm for fifty cents each. Books are duplicated for fifty cents for each fifty pages or fraction thereof. Photostats are priced at fifty cents for each ten pages or fraction thereof for any single volume. There are certain restrictions on photoduplication to protect the rights of copyright owners.

ARMY ENGINEERS TO BUILD NEW MEDICAL CENTER

According to an announcement from the Office of the Surgeon General, Technical Information Division, Washington, D. C., "the greatest medical research center in the world" will be built at Forest Glen, Maryland, just outside of Washington. Officially designated as the "Army Medical Research and Graduate Teaching Center", the project will consist of a 1000-bed general hospital, the Army Institute of Pathology building; the Army Medical Museum and Center Administration building; Central Laboratory Group buildings; and the Army Institute of Medicine and Surgery. A working library, animal farm, quarters for the staff and other buildings are included in the plans. The Center will also cooperate with the National Bureau of Standards, the National Institute of Health and the National Research Council.

TECHNICAL SECTION

THE USE OF GELATIN IN Rh TESTING AND ANTIBODY DETERMINATIONS

A RAPID TEST TUBE METHOD*

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Several methods of Rh testing are in common use, the choice of methods depending on the particular needs of each laboratory and on the quality and behavior of antisera available. The method to be described offers the advantages of speed and simplicity without the loss of accuracy and can be used with antisera containing either blocking or agglutinating antibodies.

It is well known that sera containing blocking antibodies can be used for Rh testing if the proper conditions are established for their reactions. These antisera are encountered more commonly than antisera containing agglutinins of sufficient potency for reliable Rh typing. Blocking antibodies, *i.e.*, incomplete antibodies, univalent antibodies, glutinins, produce agglutination only in the presence of complex protein systems. The first stage of the reaction, specific antigen-antibody union, proceeds as usual, but actual clumping of the sensitized erythrocytes is dependent on a protein component referred to by Wiener² as conglutinin. The conglutinin of serum or plasma is a normal blood constituent; its function in the clumping of specifically sensitized erythrocytes is lost upon dilution with any crystalline solution. Diamond and Denton¹ compared several media for agglutination by blocking antibodies and found serum albumin superior to plasma when used in the proper concentration. A 5 per cent gelatin solution which was included in their tests caused clumping of both Rh-positive and Rh-negative erythrocytes.

Gelatin solutions have been made in this laboratory which compare favorably with plasma and serum albumin in Rh blocking antibody tests. The degree of clumping produced in a gelatin medium of adjusted pH is satisfactory, and false agglutination and rouleau formation are easily overcome by saline dilution.

METHODS

The gelatin solution is made by dissolving 10 gm. of special gelatin† and 1 gm. of di-sodium acid phosphate in 90 ml. of distilled water. The preparation is sterilized in the autoclave at 121 C. for fifteen minutes and has a pH of about 6.7

* Received for publication, May 9, 1947.

† Special nonpyrogenic gelatin manufactured by U-Cop-Co, Division of Wilson and Company, Chicago 9. This gelatin is of low calcium content.

after sterilization. The solution is poured into sterile screw top bottles of the pipet dropper type and is kept ready for use at room temperature. The solution may be kept in the incubator without loss of activity to avoid solidification which may occur at room temperature. Solidified material can, however, be made ready for use by warming the bottle. Merthiolate can be used in a concentration of 1:10,000 to prevent spoilage by bacterial contamination.

The gelatin solution is used in Rh testing and in antibody determinations for sensitization to the Rh agglutinogens. Anti-Rh serums containing either agglutinins or blocking antibodies can be used in the test. Antiserums used in routine clinical testing should be of anti-Rh₀ specificity.

Procedure for Rh testing. Mix one drop of undiluted anti-Rh₀ serum and one drop of 10 per cent gelatin solution with one drop of fresh oxalated whole blood. Incubate the tube at 37 C. for one or two minutes and add 2 ml. of normal saline solution. The saline suspension formed by unagglutinated erythrocytes is smooth and homogeneous while positive reactions are easily seen by holding the tube in a slanting position over an illuminated white background. An alternate method which gives equally satisfactory results can be employed when dilute citrated suspensions of blood specimens are used for typing. Approximately 1 ml. of a 2 per cent suspension of the blood to be tested is centrifuged and the supernatant fluid removed with a pipet. The cells are resuspended in one drop of Rh₀ testing serum and one drop of gelatin solution; the mixture is incubated and diluted with saline as indicated above. The duration of incubation is not critical and the reactions do not necessarily have to be read at the one to two minute period.

Procedure for Rh antibody tests. The gelatin solution is used to intensify the reactions obtained in tests for Rh sensitization. A sensitive qualitative test which is useful for the detection of agglutinins and/or blocking antibodies is run by mixing one drop of the patient's undiluted serum or plasma with one drop of 10 per cent gelatin solution and one drop of oxalated whole blood of known Rh type. The unknown serum is tested against Rh₀-positive and Rh₀-negative blood specimens. The mixtures are incubated at 37 C. for five minutes and diluted with 2 ml. of saline before reading. Antiserums of the anti-Rh₀ type are encountered much more commonly than those of anti-Rh' or anti-Rh'' specificity. The latter types of antibodies rarely occur in the absence of Rh₀ sensitization and serums containing them are usually of anti-Rh₀' or anti-Rh₀'' specificity. In clinical testing, therefore, the use of any of the Rh₀-positive blood types should be sufficient for the detection of antibodies in the majority of serums examined. Blood specimens of Rh₁(Rh₀') and Rh₂(Rh₀'') types can be used to insure the determination of the rare antibodies and these blood types are, of course, essential in tests to determine the specificity of antibodies encountered. It is important to use fresh blood specimens in these tests. Serums demonstrated to contain Rh antibodies can then be titrated for their agglutinin and blocking antibody content.

Procedure for Rh agglutinin and blocking antibody titrations. The titrations are run by the methods given by Wiener² with certain modifications. A 3 per cent

gelatin solution, which is made by diluting 3 ml. of the buffered 10 per cent solution with 7 ml. of saline, is used in place of plasma or serum albumin in the conglutination test for blocking antibodies. It is advantageous to use larger volumes of the reagents when the tests are run with tubes of the 100 x 13 mm. size; therefore, doubling dilutions of the patient's serum in saline are made in amounts of 0.2 ml., the first tube containing undiluted serum. The serums should be titrated against cell suspensions of both Rh₁ and Rh₂ types to determine the specificity of the antibodies demonstrated. One drop of a saline washed 2 per cent group O erythrocyte suspension of known Rh type is added to the tubes which are examined for clumping by Rh agglutinins after thirty minutes of incubation at 37 C. The saline is removed after centrifuging the tubes at low speed and the cells are resuspended in 0.2 ml. of the 3 per cent gelatin solution.* Agglutination by blocking antibodies is looked for after holding the tubes at body temperature for another thirty minutes. Prior to reading the reactions, 0.2 ml. of saline is added to each tube. This does not interfere with the reaction and eliminates pseudo-agglutination.

RESULTS

The accuracy of the rapid gelatin method of Rh testing was determined with anti-Rh₀ blocking serum on a series of 500 routine blood specimens. The specimens were also typed with anti-Rh₀ and anti-Rh' agglutinating serums using washed saline cell suspensions. No false negative or false positive reactions were observed with the gelatin method and the results were in complete agreement with anti-Rh₀ specificity. The majority of anti-Rh serums obtained from women isoimmunized during pregnancy were found to be suitable for Rh₀ or Rh₀' testing by this method. Twenty out of 24 serums from this source gave satisfactory reactions for the test. All of these specimens contained blocking antibodies with or without weak agglutinins.

The 10 per cent gelatin solution was employed in qualitative Rh antibody tests on 96 serum specimens from Rh-negative women. The serums were tested against fresh oxalated whole blood of group O, Rh₁(Rh₀') and Rh₀'-negative types. In this test the tubes were examined after a five minute period of incubation. Positive reactions were obtained with 32 of the specimens examined; these serums were found to contain Rh₀ blocking antibodies in varying titers and several contained weak agglutinins. The agglutinins in certain instances were of anti-Rh' specificity and one specimen contained this type of antibody only. Gelatin intensifies the agglutination reaction produced by these antibodies as well as those of anti-Rh₀ specificity. The 64 serums which gave negative reactions were also negative in agglutination and plasma conglutination tests. The method, therefore, serves as a convenient Rh antibody exclusion test.

* Preliminary observations show that it is not necessary to remove the saline diluent used in the agglutination titration. Satisfactory results have been obtained by adding 0.1 ml. of the 10 per cent gelatin solution to each tube of the titration. This gives a final concentration of 3.3 per cent which is within the optimal range of the gelatin employed.

A 3 per cent gelatin solution, which is made from the more concentrated buffered material, was used for the titration of blocking antibodies of 20 different antisera. This solution gave essentially the same titration values as blood plasma which was used in duplicate tests on the antisera examined. The gelatin solution is clear and colorless and produces clumping of the sensitized erythrocytes which can be easily read.

DISCUSSION

It is apparent from the results obtained in this study that gelatin offers a convenient source of protein which is necessary for the completion of certain Rh agglutination reactions. Gelatin serves as a suitable plasma substitute and can be added to blocking antibody or agglutinin tests with whole blood to enhance agglutination. The material is inexpensive and gives clear, colorless heat-stable solutions which can be kept for a long time without loss of activity and which are free of interfering antibodies. The disadvantages of gelatin, *viz.*, solidification and pseudoagglutination, are readily circumvented by the methods discussed.

SUMMARY

Gelatin solutions were tested for their effect on Rh agglutination reactions. A special solution of gelatin was found to intensify agglutination by Rh blocking antibodies. Gelatin solution was used as a plasma substitute for the titration of blocking antibodies. The behavior of gelatin was utilized to devise a rapid test tube method of Rh testing. The majority of antisera encountered in routine antibody determinations were suitable for the new method of Rh₀ or Rh₀' testing.

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THE DETERMINATION OF PROTHROMBIN (TWO-STAGE METHOD) AND ANTITHROMBIN USING COMMERCIALY AVAILABLE REAGENTS*

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Present methods for the determination of prothrombin and antithrombin vary widely in different laboratories. This nonuniformity of methods with consequent lack of a basis for comparison of results appears in a large measure to be due to the inherent variability of the unstable biologic reagents used in these procedures.

In the determination of prothrombin by the two-stage method,⁵ in which the time of conversion does not form a part of the observed clotting time, the constancy of any unit of prothrombin must depend to a great extent on the reproducibility of the fibrinogen solution. Determinations of antithrombin may be affected not only by variations in the fibrinogen but also by variations in the thrombin. In reports found in the literature satisfactory evidence of the reproducibility of these reagents is usually lacking. While we were accumulating data on which to evaluate the clinical worth of these tests, it seemed worth while to explore the use of commercial reagents. The thrombin used in the present study, prepared according to the method of Seegers, was obtained from Parke, Davis & Company. Fraction I, obtained through the courtesy of Dr. E. J. Cohn from The Armour Laboratories, served as a source of fibrinogen. For the determination of prothrombin a modification of Herbert's method² was employed and for the determinations of antithrombin, a modification of the method of Astrup and Darling.¹

METHOD FOR THE DETERMINATION OF PROTHROMBIN

Reagents

(a) *Physiologic saline solution.* Sodium chloride was dissolved in triple distilled water to make a 0.9 per cent solution and the solution was sterilized.

(b) *Imidazole buffer.* Eighty-six hundredths of a gram of imidazole was dissolved in 45 cc. of tenth normal hydrochloric acid and the volume was made up to 50 cc. with distilled water (pH 7.2).

(c) *Calcium chloride-saline solution.* One-tenth of a gram of calcium chloride (anhydrous) was dissolved in 200 cc. of physiologic saline solution (a).

(d) *Thromboplastin.* Fifteen hundredths of a gram of acetone-extracted rabbit brain (Bacto thromboplastin obtained from Difco Laboratories, Inc., Detroit, Michigan) was ground in a mortar with 15 cc. of calcium chloride-saline solution (c) and the mixture was allowed to stand, with intermittent stirring, for ten minutes. It was centrifuged for two minutes at 1000 R.P.M. in a No. 2 International centrifuge. The supernatant fluid was removed and diluted with twice its volume of calcium chloride-saline solution (c). This extract was made fresh daily.

* Received for publication, June 28, 1947.

(c) *Solution of acacia.* Twelve per cent acacia was dissolved in 1.1 per cent disodium acid phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) to give a solution of pH 7.2. Much of the calcium of the gum precipitated as tribasic calcium phosphate and was removed by filtration.

(f) *Fibrinogen.*

(1) *Stock solution.* Four per cent Fraction I (bovine origin, containing about 40 per cent sodium citrate) was dissolved in saline solution, added to a series of tubes and placed at -20°C . In the frozen state, this solution maintained its activity for five days.

(2) *Dilute solution.* A 1 per cent solution of Fraction I was prepared from the stock solution (1). The activity of the stock solution was checked in the antithrombin determination. Only solutions of comparable activity were used. (The frozen fibrinogen was melted by placing it in a 37.5°C . water bath for three minutes.) Immediately before use the 1 per cent solution of fibrinogen was diluted with equal parts of solution of acacia (c). Thus the final concentration was equal to 0.5 per cent of the Fraction I preparation.

(g) *Thrombin.* The contents of one ampule of dried thrombin (bovine origin: Parke-Davis assay = 5000 Iowa units) was dissolved in physiologic saline solution (a) to produce a solution having an activity of 100 units per cubic centimeter.

Defibrination of plasma. One cubic centimeter of plasma from the blood collected as for the Quick prothrombin test was defibrinated by adding 0.1 cc. of thrombin (10 units), allowing it to stand for fifteen minutes and expressing the serum from the fibrin clot by means of an applicator.

Dilution of plasma. Plasmas from normal persons were diluted 1:25 with physiologic saline solution (a). Plasmas from patients were diluted 1:25 or 1:10 depending on the degree of prothrombin deficiency present.

Procedure

Five-tenths of a cubic centimeter of imidazole buffer and 1.0 cc. of thromboplastin (containing calcium) were added to 0.5 cc. of diluted plasma. A stop watch was started immediately after addition of the thromboplastin. The mixture was allowed to incubate at room temperature and samples were taken at intervals of one minute for clotting tests beginning after three minutes of incubation. Two-tenths of a cubic centimeter was measured into a tube, placed in a water bath at 28°C . and 0.2 cc. of fibrinogen-acacia solution was added. The time required for the clotting of the fibrinogen-acacia solution was noted. The end point was chosen as that point at which there was definite fibrin formation. The shortest clotting time obtained with the samples taken from the incubation mixture was used in the determination of the amount of prothrombin present. The prothrombin was expressed in units essentially comparable to the units as defined by Warner, Brinkhous and Smith.* The fibrinogen-acacia solution used was standardized against varying concentrations of Parke-Davis thrombin. When the clotting times obtained were plotted against concentrations of thrombin on logarithmic paper a straight line was obtained over a range of ten to forty seconds (Fig. 1). From this curve the prothrombin activity of a particular diluted plasma could be determined by noting the concentration of thrombin giving the same clotting time on addition of the fibrinogen-acacia solution. The total

* One unit of prothrombin is the amount which when converted will form 1 unit of thrombin. One unit of thrombin is the amount required to clot 1 cc. of a standardized fibrinogen in fifteen seconds. The latter unit is frequently referred to as the Iowa unit.

prothrombin per cubic centimeter of plasma was then calculated by multiplying the prothrombin activity of the diluted plasma by the dilution factors as follows:

- 1.2—oxalate
- 1.1—defibrination
- 25—dilution of plasma
- 4—dilution by incubation mixture.

By this procedure the average of 30 different normal subjects (staff members or blood donors) was 317 prothrombin units per cubic centimeter of plasma (range

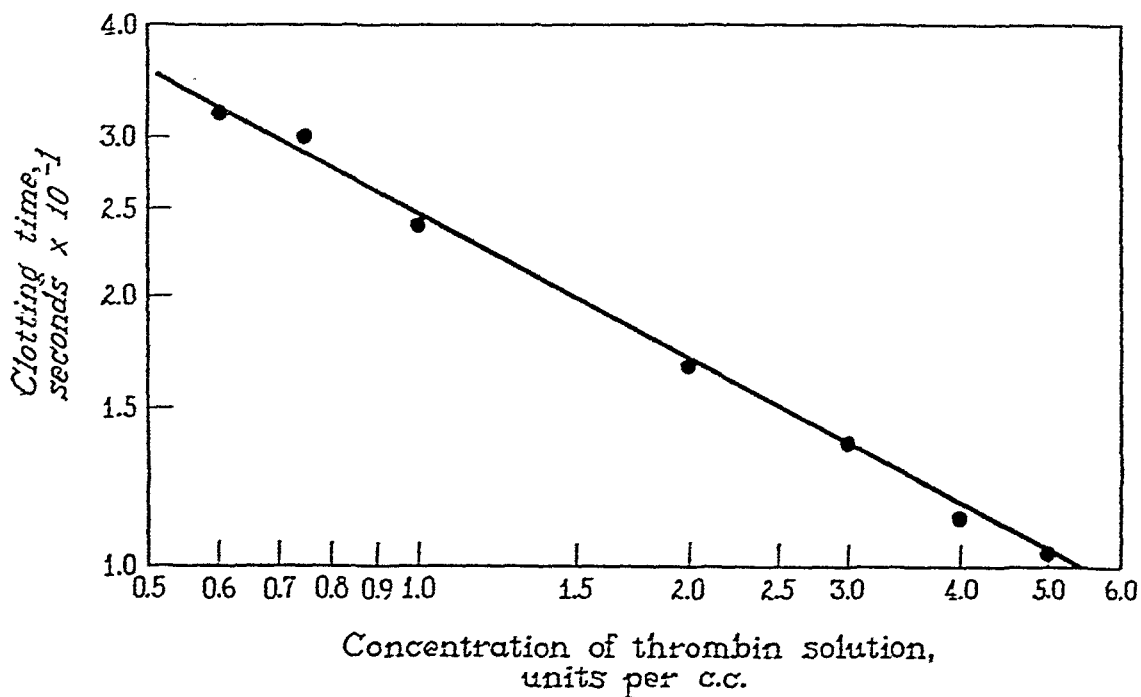


FIG. 1. Relationship between thrombin concentration and clotting time when 0.2 cc. of fibrinogen-acacia mixture was added to 0.2 cc. of thrombin solution.

from 244 to 378 units). Percentage of normal was calculated from this figure. When a series of dilutions from 1:10 to 1:25 was run on a normal plasma, the results were in good agreement.

METHOD FOR THE DETERMINATION OF ANTITHROMBIN

Thrombin. Thrombin was dissolved in a volume of saline solution to give a solution containing 600 units per cubic centimeter. Solutions equivalent to 200 units per cubic centimeter and 6 units per cubic centimeter respectively were prepared from the stock solution.

Fibrinogen. Two per cent Fraction I was prepared from the 4 per cent stock solution of Fraction I (see method for prothrombin). This solution was standardized against the thrombin solution in the following manner: 0.5 cc. of thrombin (3 units) was placed in a tube in the 25 C. water bath and 0.5 cc. of 2 per cent fibrinogen was added. The time required for the formation of a clot was recorded.

Procedure

Five-tenths of a cubic centimeter of serum was placed in a 50 cc. centrifuge tube. (When the coagulation time obtained was more than 40 seconds, the test

was repeated using 0.3 cc. of serum.) Five-tenths of a cubic centimeter of thrombin solution (100 units) was added and a stop watch was started. The tube containing the mixture was placed in a 37.5 C. water bath. After ten minutes' incubation, 9.0 cc. of saline solution was quickly added and 0.5 cc. of diluted mixture removed and placed in a tube in the 28 C. water bath. Five-tenths of a cubic centimeter of 2 per cent fibrinogen was promptly added and a stop clock was started. The time for the formation of a clot was recorded.

Under the conditions of the antithrombin assay, the clotting time was found to be a linear function of thrombin concentration over the range of fifteen to fifty seconds. For each series of determinations, the fibrinogen and thrombin solutions used were tested against each other. The thrombin in a concentration of 3 units per cubic centimeter clotted the fibrinogen in sixteen to eighteen seconds. This control time actually observed on each occasion was used in calculating the amount of thrombin destroyed by the serum as follows:

$$\text{Thrombin units destroyed} = 100 - \frac{\text{Clotting time of control}}{\text{Clotting time of unknown}} \times 60.$$

When antithrombin determinations were done using amounts of from 0.1 to 0.5 cc. of normal serum made up to 0.5 cc. with physiologic saline solution, the relationship between the amount of serum and the amount of thrombin destroyed was never linear. When the logarithms of these quantities were plotted against each other a straight line was obtained, both with normal serum and with serum of elevated antithrombin activity (Fig. 2). Astrup and Darling calculated antithrombin activity from thrombin destruction by a linear formula but stated that the values for the lowest and highest amounts of plasma or serum often deviated from this rule. A dilution curve of a normal serum of average activity was arbitrarily selected as standard. Five-tenths of a cubic centimeter of this serum destroyed 62 units of thrombin under the conditions of assay. Serums from 47 normal persons (staff members or blood donors) destroyed from 51 to 66 thrombin units. From the logarithmic dilution curve this can be expressed as antithrombin activity corresponding to that of from 0.35 to 0.57 cc. of the standard serum. The average of all these normals was 0.48 cc. of the standard; the nearness of this value to 0.5 shows that the standard had an activity very close to the average of the group of normals. Antithrombin activity of pathologic serums was always referred to the same standard and then converted to percentage of normal as follows:

Per cent antithrombin

$$= 100 \times \frac{\text{Amount of standard serum corresponding to activity of 0.5 cc. of unknown serum.}}{0.48}$$

If a fraction of 0.5 cc. of the unknown serum was used, the factor 0.48 was of course multiplied by that fraction. This calculation ignores possible variations in the slope of the dilution activity curves. Even in specimens of unusually high

antithrombin activity such variations do not appear to be large. While no explanation of the dilution curve is offered at present, it should be noted that it necessitates working in a range of activity where more than half of the total thrombin substrate is destroyed, contrary to the usual practice in assays of this type. When smaller fractions of thrombin are destroyed, not only is there relatively little change in thrombin destruction on varying the amount of serum but

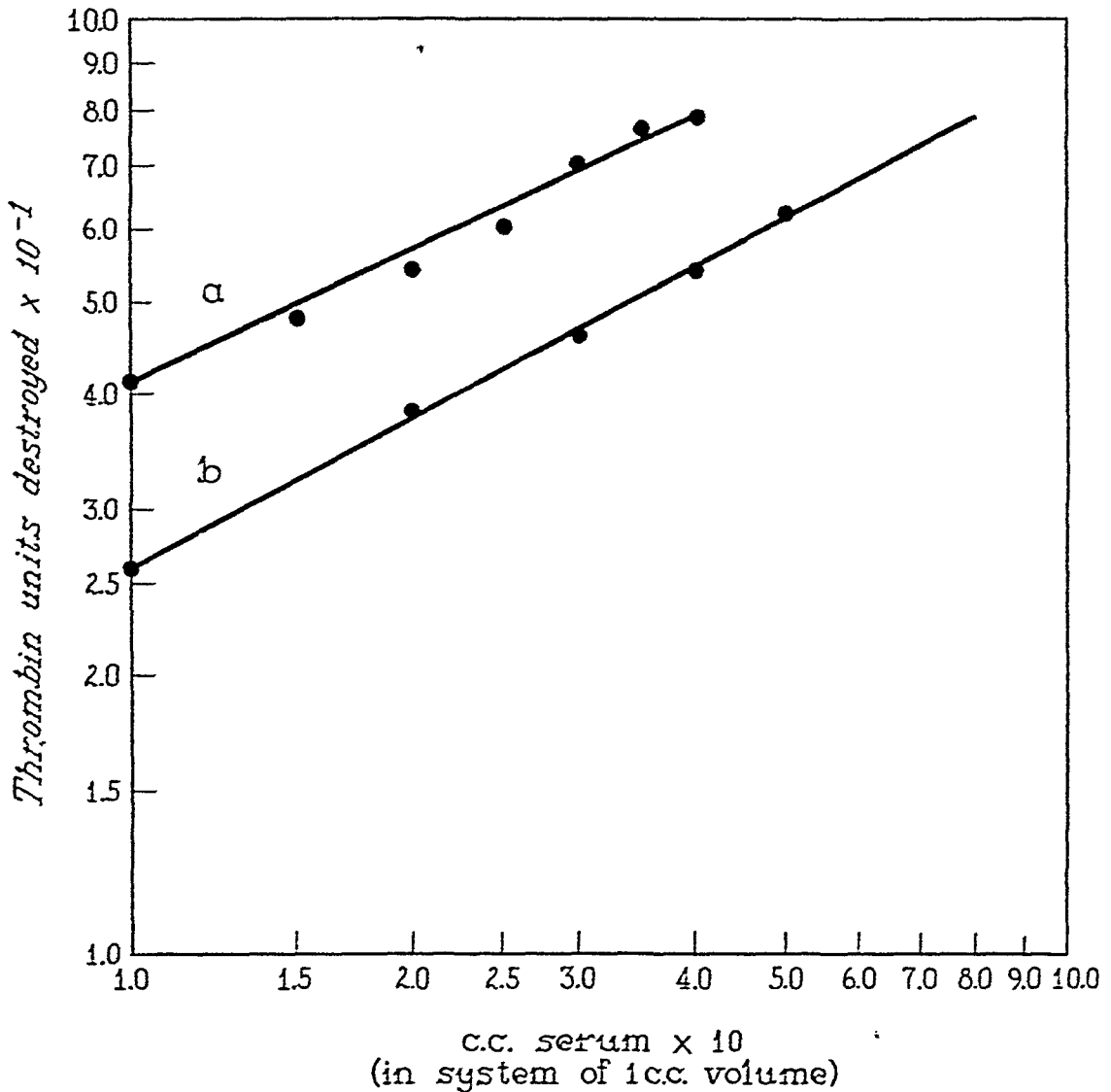


FIG. 2. Relationship between thrombin destruction and concentration of serum. *a*. Serum with elevated antithrombin. *b*. Normal serum.

there is even less change in the clotting time actually measured, as "thrombin destroyed" is measured by difference.

COMMENT

It is recognized that solutions prepared from dried thrombin and fibrinogen are not necessarily of the same activity as those prepared from fresh thrombin and fresh fibrinogen and that species differences must be taken into account. How-

ever, fresh preparations of thrombin and fibrinogen may also vary in their activities. The thrombin and fibrinogen solutions as prepared were of comparable activity and it was felt that information as to relative amounts of prothrombin and antithrombin was valid.

The methods as outlined were used in a study concerning the variations in prothrombin and antithrombin in patients who were receiving Dicumarol³ and in patients with thrombosing tendencies.⁴ The employment of the commercial reagents greatly facilitated the use of these methods as clinical tests.

SUMMARY

Methods for the determination of prothrombin (two-stage) and of antithrombin using commercially available reagents have been described. Solutions of thrombin and fibrinogen, as we prepared them, have possessed similar activity.

The use of commercial reagents has simplified the procedures for the determination of prothrombin by the two-stage method and of antithrombin.

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AGGLUTINATION TUBE RACKS FOR USE WITH THE MICROSCOPE*

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Most blood-group serologists recommend that agglutination tests be performed in small test tubes rather than on slides. Evaporation occurs but slowly from tubes, and since the suspensions may be examined over longer periods, numerous tests may be carried out at one time. Needless to say, the use of test tubes also permits centrifugation and incubation in a water bath or refrigerator. For such tests, 75 x 10 mm. test tubes with lips are ideally suited, since their diameters are sufficiently small to permit direct microscopic examination of the cell suspensions through the wall of the test tube.

The apparatus described in this note has been found to facilitate greatly the handling and reading of such tubes. It has been used in this laboratory in connection with genetic studies, where several cell suspensions are tested simultaneously against a battery of several serums, and it is particularly recommended to those who are engaged in blood typing studies of a similar nature. The increased demand on hospital technicians for Rh titrations and tests with several varieties of serum, however, suggests that the equipment might also be of value in routine clinical work.

Racks. The racks are of simple one piece construction (Fig. 1). They consist of wood (soft pine) or transparent plastic blocks ($12\frac{1}{2} \times 1\frac{1}{4} \times \frac{3}{4}$ inches), each having a series of 20 vertical holes. The holes should be just large enough to freely admit the tubes. Since "75 x 10 mm." tubes of various manufacturers vary appreciably in diameter, it is well to test the recommended $\frac{27}{64}$ inch hole against the supply of tubes at hand. Also, it might be mentioned that the tubes of some manufacturers are much superior to others, having stronger walls and lips, with evenly-annealed bottoms which produce a minimum of optical distortion.

When the racks are supported in a frame or tray, the tubes are held upright in a rigid and compact alignment, which facilitates the accurate pipeting of cell suspensions and testing fluids. When the racks are lifted from the tray, the tubes are suspended by their lips. In this way the contents of the tubes may be checked at each stage in the serologic procedure. Mixing can be accomplished by tapping the end of a single tube or by shaking the entire rack or the entire tray.

Water bath supports. A support to hold 10 racks (200 tubes) is shown in Fig. 2. This consists of a bottomless tray or frame, the sides of which are $\frac{1}{4}$ inch in thickness to accommodate the slots at the ends of the racks. Each rack is therefore securely supported but is free to be shifted from one position to another

* Studies in human heredity at the University of Michigan are supported by research grants from the Board of Governors of the Horace H. Rackham School of Graduate Studies. Received for publication, May 3, 1947.

within the frame, so that the order of the racks may be easily changed. If the frame is supplied with legs (machine bolts make convenient legs in the case of a wooden frame), the support may be set into a water bath in such a way that only the ends of the tubes are immersed. After incubation, a single rack or the entire tray may be removed from the bath, and the bottoms of the tubes carefully dried with a cloth before viewing under the microscope.

Microscope stand. The principal advantage of the single-row rack is that it enables a series of 20 tubes to be examined microscopically without removing the tubes from the rack. For this purpose a special stand is needed to support the

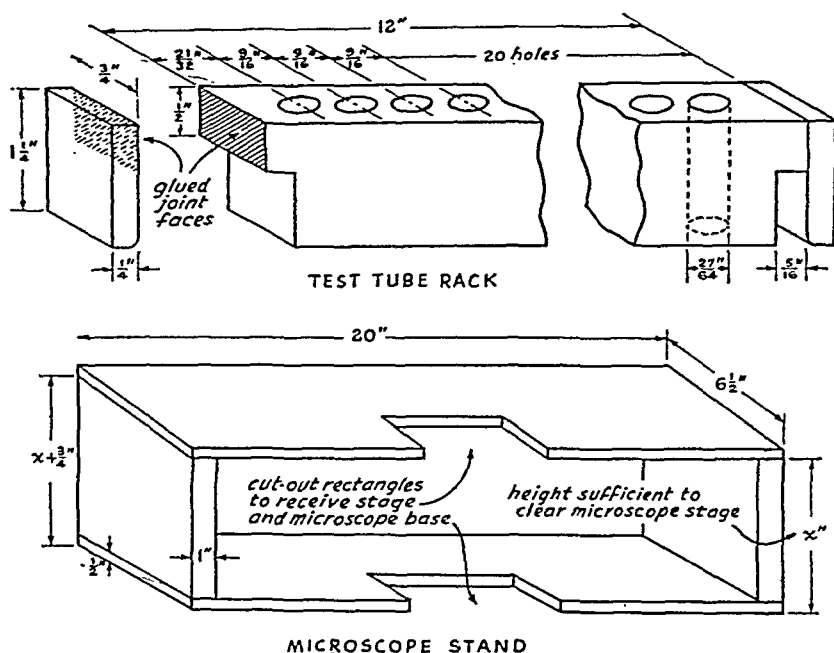


FIG. 1. Construction of test tube rack and microscope stand.

rack above the microscope stage in a nearly horizontal position. The rack is pushed from right to left, bringing one tube after another into view under the objective. Figure 2 shows a supporting shelf which has been hinged onto a viewing box containing a fluorescent bulb, which serves as the microscope lamp. A more simply constructed stand for use with an ordinary microscope lamp is shown in Figure 1. Either a compound microscope with a low power objective or a wide-field binocular microscope with medium and high power objectives may be used.

Figure 2 shows the equipment in use. In addition to the factors mentioned above, the following advantages are afforded. Since the tubes need not be removed from the racks (unless they are to be centrifuged), there is no necessity for

marking them and no chance for misclassification due to misplacement of the tubes. The first tube in each rack may be marked or a labeled tube containing the patient's cell suspension may occupy one hole, serving to identify the rack. Standard positions may be adopted for the various test serums (*e.g.*, anti-A₁, anti-A, anti-B, anti-M, anti-N, anti-C, anti-D, anti-E, anti-c). Each rack holds

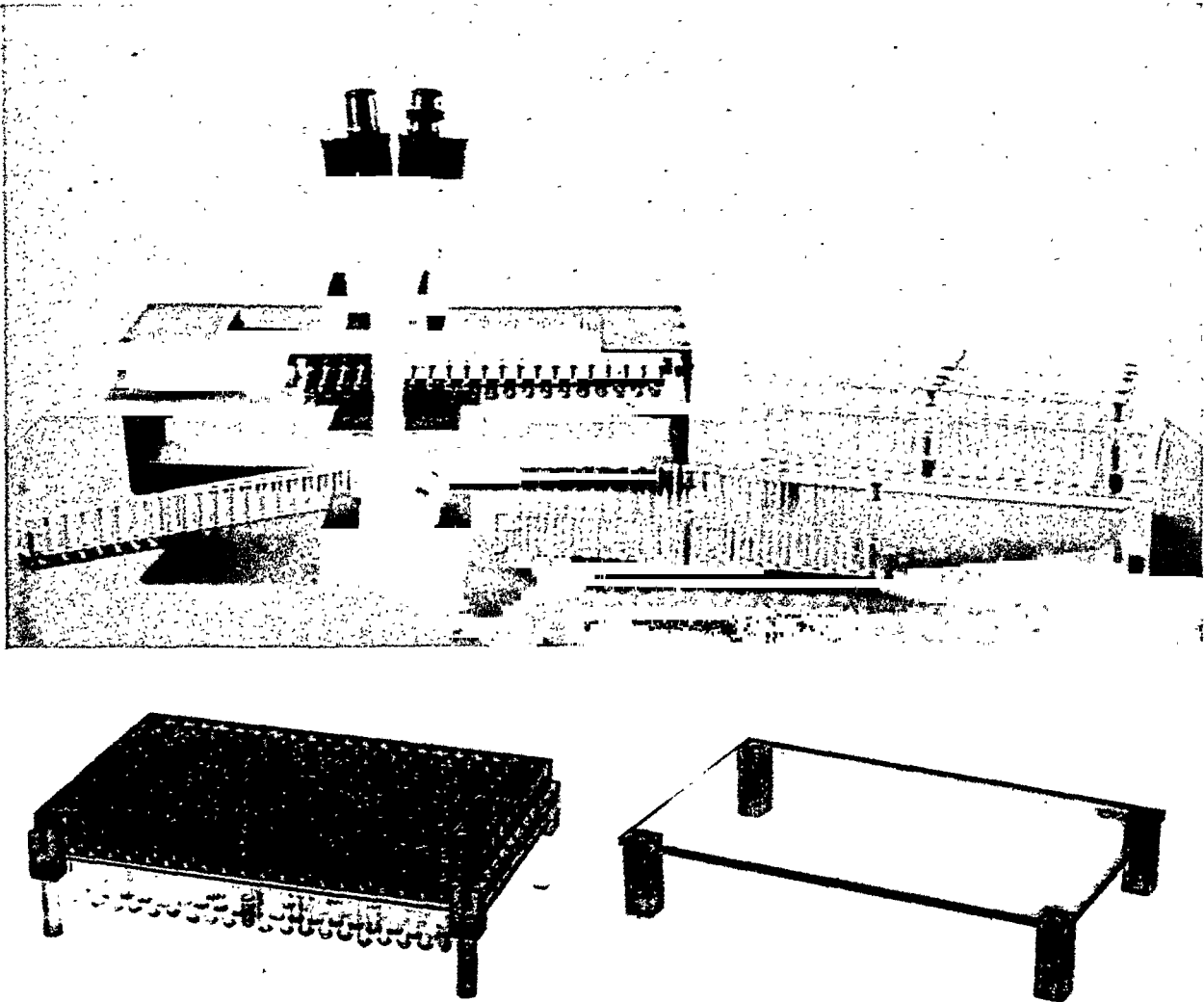


FIG. 2. Agglutination tube racks and supports in use with microscope.

a sufficient number of tubes to allow titrations of serums to be carried beyond 1:512, as is sometimes necessary.

The fixed position of the test tube under the microscope objective frees both hands of the technician for manipulation of the microscope and recording of readings. Being much less bulky than standard serologic racks, the special equipment conserves much space on the laboratory table or in the water bath.

SIMULTANEOUS HISTOLOGIC FIXATION AND GROSS DEMONSTRATION OF CALCIFICATION*

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It may sometimes be desirable to use a reagent which grossly demonstrates areas of calcification, as in lymph nodes or in fat necrosis, and which at the same time is a good general fixative, permitting the use of trichrome stains without the necessity of using additional procedures, such as refixation of sections. Hitherto, for the gross demonstration of calcium soaps in fat necrosis, formalin-fixed tissues were incubated for twenty-four hours at 37 C. in Weigert's glia mordant, a mixture containing copper acetate. As a result of the combination of copper with fatty acids, the areas of calcified fat necrosis stained green. This reaction could also be obtained in calcified atheromatous plaques of the aorta.² As far as could be ascertained, this method has never been used for the demonstration of other kinds of calcification, such as calcified bone.

It is evident that this method entails the well known disadvantages of formalin fixation; moreover, two different fluids have to be used. Excellent histologic fixation and simultaneous distinct visualization of calcified structures can be obtained by the use of the fluid of Bouin-Hollande. The formula of this fixative, which seems to be almost entirely unknown in the United States, is as follows:¹

Distilled water, 100 ml.; copper acetate, 2.5 gm.; picric acid, 4 gm.; formaldehyde, U.S.P., 10 ml.; glacial acetic acid, 1.5 ml.

The copper acetate is dissolved in water without the use of heat and picric acid is slowly added under constant stirring.

When the picric acid is dissolved, the solution is filtered and the formaldehyde and acetic acid are added. The prepared solution keeps indefinitely. Tissues are left in this fixative from eight hours to three days; after the fixation is complete they are washed for several hours in two or three changes of distilled water.

Calcified structures, such as bone, calcified necrotic tissue and calcified fat necrosis are stained dark green on a pale green background. I have also obtained distinct visualization of calcified cartilage and bone in human embryos, and of the remnants of bone in the process of bone destruction. As has already been stated, the fixation of histologic details is excellent.

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* Received for publication, June 28, 1947.

A SPRING-ACTUATED MULTIPLE PUNCTURE APPARATUS FOR BCG VACCINATION*

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One was led to believe that Rosenthal's³ multiple puncture method of BCG vaccination had been sufficiently simplified by an 8-needle vaccination apparatus¹ to make the method universally available. At the BCG clinic in Bergen, Norway, it had been used on 3727 tuberculin-negative infants and adults vaccinated with a 50 mg. per ml. concentration of BCG vaccine. When retested two months later with 1.0 mg. of old tuberculin intracutaneously, 97.5 per cent had become positive and none had developed local or adjacent abscesses. Shortly afterwards it was learned, however, that some physicians had experienced considerable difficulty in obtaining vaccination papules with the apparatus. On further investigation, it was ascertained that most of the difficulties were due to a highly variable technic. In order to standardize the decidedly advantageous and nonabscess-producing, multiple puncture method of BCG vaccination, a 40-needle, spring-actuated vaccination apparatus² built on the piston-release principle was developed. Up to 1946, more than 3000 tuberculin-negative persons were vaccinated by means of this apparatus and 98 per cent of them became positive within two months. Again it was noted that there was a complete absence of local suppuration or abscesses.

Following the intracutaneous injection of from 0.05 to 0.1 mg. of BCG, the incidence of blemishing abscesses is seldom below 10 per cent and some workers report a frequency of 50 per cent or more.⁴ The multiple puncture method is, therefore, a definite and important advance in the popularization of BCG vaccination against tuberculosis.

Figures 1 and 2 depict the apparatus which is 12.5 cm. long and 4.3 cm. wide. In Figure 1, the 40 needles are drawn up into the housing and the cock is lifted. In Figure 2, the needles protrude from the headplate after the release of the spring-actuated piston. The needles are ordinary, noncorrosive, phonograph needles soldered into a circular brass disc. The actuating piston operates through the long axis of the apparatus, with two guides that align the needles with the forty perforations in the headplate. The actuating piston is pulled back into a locked position under spring tension. The piston is released by pressing the trigger and the needles are thrust through the apertures in the headplate. The distance that the needle points extend beyond the surface of the headplate is adjustable. Usually 2 to 3 mm. is adequate for penetration of the epidermis. To facilitate the manipulation of the spring-actuated apparatus, which necessitates a moderate pressure against the skin during the vaccination process, finger grips

* Received for publication, June 23, 1947.

are placed on each side of the instrument. The apparatus is made of nickel-plated brass and weighs 205 gm.

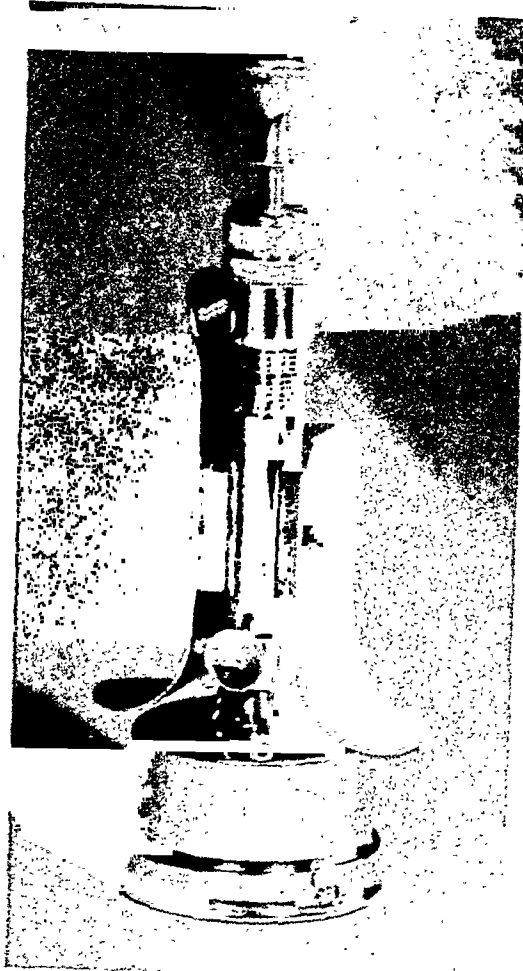
The following technic has been found practical for individual or mass BCG vaccination. An area of skin, 10 x 10 cm., on the outer surface of the upper arm or thigh is cleaned with ether. A piece of thin paper or cellophane, 4 x 4 cm., sterilized for fifteen minutes at 100 C., is moistened on both sides with 20 mg. per ml. of BCG vaccine in a sterile Petri dish and is placed on the ether-cleaned skin area. Care should be taken to hold the arm or thigh in a horizontal plane in order to retain most of the vaccine on the paper. The cock of the apparatus is drawn up. The skin is held taut by grasping the arm or thigh on opposite sides. The headplate is pressed against the paper and the trigger is pushed. The needle plate descends with some force and the needle points become coated with the vaccine as they perforate the paper and enter the epidermis to a depth of 1 to 3 mm. The apparatus is removed and the headplate with protruding needles is placed on a thick pad of cotton saturated with 70 per cent alcohol. The paper is removed from the arm after one or two minutes. No bandage is necessary over the vaccinated area. Small petechial bleeding may be seen in the punctures when the skin is stretched. Excessive spontaneous bleeding should be avoided; this can be controlled readily by adjusting the extension of the needles to 2 or 3 mm. beyond the headplate.

Red papules, 1 to 2 mm. wide, and slightly elevated, appear after one week and reach maximum development within one month after vaccination, when desquamation sets in. Healing is complete within six to eight weeks without visible scars or pigmentation. Abscess formation is exceedingly rare with the multiple puncture method and is usually associated with questionable positive tuberculin reactions (allergy) at the time of vaccination. Figure 3 shows the pattern of forty papules three weeks after vaccination.

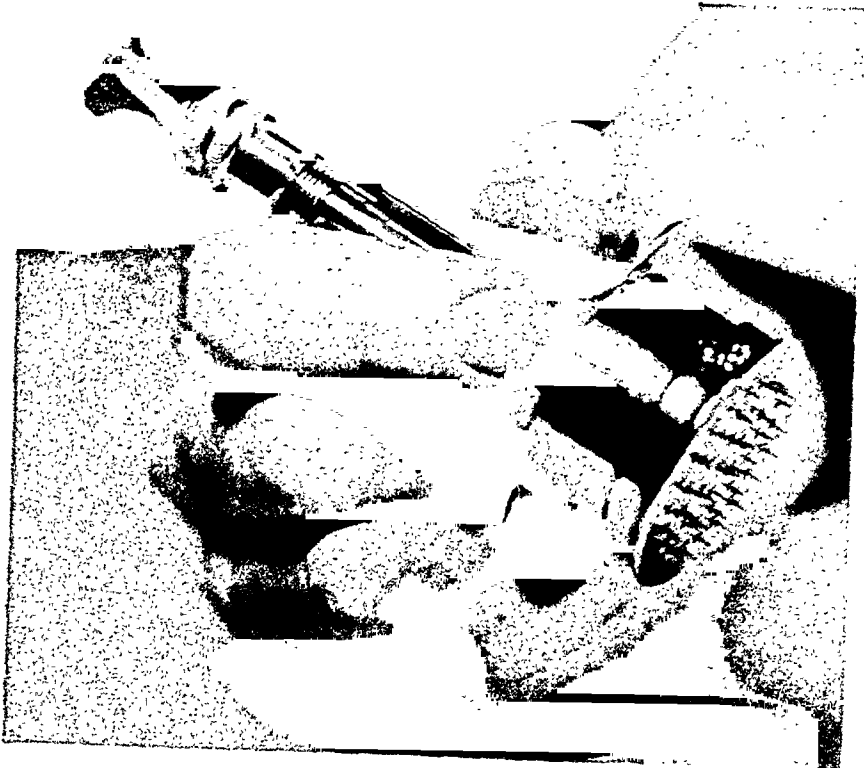
When larger groups of tuberculin-negative persons are to be vaccinated with BCG, the procedure may be facilitated by placing from ten to twenty pieces of sterile paper in tile formation in the 20 mg. per ml. BCG vaccine in a Petri dish, leaving an edge of 1 to 2 mm. that can be grasped with forceps.

A serious handicap in mass vaccination with the automatic apparatus is the loss of time in sterilizing it between each vaccination. The following short cut has been found practical and no cross infections have been encountered. Between each vaccination, the headplate with the needle points protruding is placed on a thick pad of cotton saturated with 70 per cent alcohol. After two to three minutes, the headplate is passed quickly through a flame until the alcohol is evaporated, taking care not to soot or overheat the needles. An alternative is to immerse the headplate with the needle points protruding in boiling water for fifteen seconds, followed by ten to fifteen seconds in cold sterile water. The apparatus is then placed on a pad of sterile absorbent cotton to remove excess water. If two or three automatic instruments are available, more time can be allowed for

Fig. 1. Spring-actuated multiple puncturé apparatus for BCG vaccination.
Fig. 2. Same as Fig. 1, showing the 40 protruding needles.



1



2

the sterilization of each apparatus. With adequate technical assistance, it has thus been possible to vaccinate singlehandedly 200 persons per hour. For individual vaccination, the apparatus should be sterilized before and after its use, preferably with dry heat (100 C. for fifteen minutes), since boiling will corrode the internal mechanism.



Fig. 3. The pattern of 40 papules three weeks after vaccination.

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STAINING INTESTINAL PROTOZOA WITH IRON-HEMATOXYLIN-PHOSPHOTUNGSTIC ACID*

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Heidenhain's iron-hematoxylin method is widely used in examinations for intestinal protozoa both because of the permanence of the stain and the clarity with which fine nuclear structure is demonstrated. The original method is time-consuming but many of the modifications shortening it sacrifice definition or permanence. The most difficult procedure, and the crucial one, is differentiation of the overstained slide. This requires control by microscopic examination, and considerable experience is necessary for success. Various alternative procedures have been suggested to allow greater latitude in this step, such as differentiation by dilute iron alum or picric acid. Other methods time the staining procedure to obviate differentiation. One of these² that gives satisfactory results is, however, inconvenient because of the heating advised. Another^{1, 3} omits mordanting and uses Mallory's phosphotungstic acid hematoxylin which is self-differentiating. This method gives excellent uniform nuclear detail but poor cytoplasmic definition.

Ziegler⁴ has described a method for differentiating hematoxylin-stained tissues with phosphotungstic acid. We have found that this procedure can be applied to the differentiation of intestinal protozoa with highly satisfactory results. The phosphotungstic acid does not completely decolorize the protozoa since it seems not to remove the stain from the parts of the microorganisms selectively stained after mordanting. Uniform permanent preparations can be secured by this method even by unskilled technicians, and it is rapid. The following schedule summarizes the method.

IRON-HEMATOXYLIN-PHOSPHOTUNGSTIC ACID METHOD FOR STAINING INTESTINAL PROTOZOA

Film preparations must be thin, wet, fresh, and must not be allowed to dry throughout the procedure.

1. Fix in Schaudinn's fluid in saline solution, five minutes.
2. Remove residual mercury in 70 per cent alcohol (with iodine added to obtain "port-wine" color), one minute.
3. Remove residual iodine in 70 per cent alcohol, two changes, one-half minute each.
4. Mordant in 4 per cent aqueous ferric ammonium sulfate, three to five minutes.
5. Wash in running tap water, one minute.
6. Stain in week-old 0.5 per cent aqueous hematoxylin, one minute.

* Received for publication, May 23, 1947.

7. Remove excess stain by dipping in water.
8. Differentiate in 2 per cent aqueous phosphotungstic acid, two minutes or more.
9. Blue the hematoxylin in tap water, one minute.
10. Dehydrate in 95 per cent and in absolute alcohol, one minute each.
11. Clear in xylol, one minute.
12. Mount in dammar.

PROCEDURE

Fixation. Fresh fecal films prepared on slides or cover slips should be fixed while wet. Cover slips require more delicate handling and so encourage thin preparations, which are easier to examine. Slides, however, are more durable for routine use. Fixation while the film is wet and fresh is of considerable importance, because drying results in degeneration and distortion of the protozoa. Drying at any subsequent step is similarly damaging. A large number of fixatives have been employed, but protozoologists show a marked preference for Schaudinn's fluid. The saline modification used by James¹ in his exhaustive study, is particularly good. This consists of 2 parts of saturated solution of mercuric chloride in physiologic salt solution and 1 part of absolute alcohol to which from 2 to 5 per cent of glacial acetic acid is added just before use. Artifacts are more frequent when fixatives are made in water rather than saline solution, particularly when trophozoites are present. *Dientamoeba fragilis* can often be fixed in the saline mixture when the aqueous solution fails. Fixation time is of comparatively little importance provided a minimum time is attained. From five to ten minutes suffice at room temperature; longer periods will not improve results. Fixation may be speeded by raising the temperature to 60 C., but at the risk of producing some artifacts, which are not ordinarily likely to confuse those working with feces, but which are pronounced in cultured forms. Short fixation will impair the permanence of the stain and decrease its definition. It should always be remembered that no stain can compensate for thick films, degenerated forms, or poorly fixed material.

Mercuric chloride present in the fixative must be removed from the slides by treatment for from one to three minutes with 70 per cent alcohol made up to port-wine color by the addition of iodine. It may be removed equally well, although less rapidly, by several changes of 70 per cent alcohol. The iodine, in turn, must be removed by further 70 per cent alcohol in one or two changes of one-half minute each. One of the chief advantages of using port-wine alcohol is that it discolors the succeeding alcohol step, giving warning that the reagent is becoming contaminated. If one is hurried, the steps required to remove the mercury may be ignored, but the resulting preparations are not uniform.

Mordanting. The important step of mordanting is performed with from 3 to 5 per cent violet crystals of ferric ammonium sulfate in distilled water. Upon this treatment depends the selective action of hematoxylin used later. Here, too, a definite minimum of time must be met for good results. For fecal films, this appears to be from three to five minutes. A longer period, while not harmful

to staining, generally exerts a macerating effect on the film. It is frequently claimed that the mordanting is better if allowed to act over-night. This may be true of tissue sections in which somewhat different stain values are sought, but it does not apply to fecal films. Ferric ammonium sulfate stock solution keeps well. It degenerates with use, however, and should be replaced daily. The mordant must be washed out of the film or it will blacken the hematoxylin. One minute's washing in running tap water will accomplish this.

Staining. The film is placed for one minute or more in hematoxylin solution to overstain the protozoa, for which the mordant has given it selective affinity. To do this, a surprisingly short time suffices. It is essential that one realize the aim of this step. It has been timed in other methods either to eliminate differentiation or to facilitate the action of the differentiating solution used later. For instance, if the mordant or picric acid is used for differentiation, the staining must be prolonged. Most of the alum hematoxylins give poor results at this step, regardless of time. One week old aqueous 0.5 per cent hematoxylin will be found quite satisfactory when used for one or two minutes. If this solution is to be used only occasionally, it can best be prepared by having at hand a 10 per cent solution of commission-certified hematoxylin crystals in 95 per cent alcohol. This stock should be kept in a well stoppered bottle for a day, after which 5 ml. should be diluted to 100 ml. with distilled water. The diluted stain should give a violet color when a drop is added to alkaline tap water. Fresh preparations require three to five minutes' staining time. In general, riper hematoxylin gives darker results in shorter time, but this factor is not nearly so important in staining protozoa as in tissue. Protozoan cysts usually require slightly longer staining periods than do trophozoites.

Differentiation. The stained preparation is next differentiated two minutes or more in 2 per cent aqueous phosphotungstic acid solution. If the film is thick or heavily stained, a longer time is required than when a light overstain has been obtained. In any case, the differentiation is automatic and self-limiting; films will not be decolorized whether left two minutes or two hours. The background is progressively destained leaving the protozoa unchanged, a fact which is noteworthy in preparing films containing smaller cysts for teaching purposes. For such preparations, one hour in phosphotungstic acid is recommended.

Washing. Following differentiation, the film is washed in tap water or dilute alkali to blue the hematoxylin. It is then dehydrated through 95 per cent and absolute alcohol to xylol and mounted in dammar. Fogging will result if dehydration is incomplete.

SUMMARY

A modification of Heidenhain's iron-hematoxylin method of staining intestinal protozoa is presented. The basic change is the application of 2 per cent aqueous phosphotungstic acid for differentiation. Uniform permanent film preparation of fecal specimens may be made rapidly by the modified procedures, even by persons of limited experience.

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RECTAL SWAB METHOD FOR ISOLATING *EBERTHELLA TYPHOSA*.

ITS USE IN A TYPHOID EPIDEMIC*

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In January 1945, during the waning course of a typhoid epidemic among civilians in Prato, Italy, we had the opportunity to use the rectal swab method for isolating *Eberthella typhosa*. Cultures were made by the rectal swab method in two classes of patients: (1) new patients suspected of typhoid fever who were admitted daily to the civilian hospital; and (2) a group of about 250 former patients who had been discharged to their homes without an attempt to determine their status as carriers.

METHOD

The patient was placed on a table or bed on his side, with thighs and knees sharply flexed so that the knees were close to the chest. One gloved hand of the operator raised the uppermost buttock bringing the anal orifice into view. A cotton tipped swab moistened in selenite F enrichment broth for lubrication was gently inserted into the rectum of the patient for a distance of two or three inches and given one or two twists; deep breathing by the patient aided the insertion. The small wet swab could be inserted easily in this manner even with patients who had hemorrhoids. Immediately on removal, the swab was dropped in selenite F broth contained in test tubes plugged with cotton. The cultures, after transport to the base laboratory, were incubated over-night, then shaken and plated on desoxycholate citrate agar. Colorless colonies appearing on incubation were isolated and identified by a simplified serologic *Salmonella* typing method shown to us by Captain D. W. Bruner, V.C., of the Fifteenth Medical General Laboratory.

RESULTS

Cultures were taken by the rectal swab method from each of about 100 febrile civilian patients with suspected typhoid fever and *E. typhosa* was isolated from 89 of these patients. In general, a positive culture was obtained from the first rectal swabbing of patients with typhoid fever, but occasionally two or three swabs had to be made. Rectal swabs were also taken from each of 250 former patients who had been discharged to their homes and among these patients, ten convalescent carriers of typhoid bacilli were found.

Other enteric pathogens found were *Salmonella typhimurium* and a few strains of dysentery bacilli (Sonne, Boyd 88, Boyd 119 and ambigua). Selenite F enrichment is toxic to some strains of dysentery bacilli,^{1, 2} so that if these bacteria are sought *direct plating* of the rectal swabs on selective medium is essential.

* Received for publication, April 30, 1947.

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SUMMARY

When typhoid patients are constipated, or in typhoid epidemics, rectal swabbing followed by enrichment and plating of cultures on selective medium is a practical procedure for the isolation of *Eberthella typhosa*.

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UTILIZATION OF VITAMIN B₁₂ CONJUGATE IN PERNICIOUS ANEMIA*

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The first observations regarding the utilization of Vitamin B₁₂ orally by anemic subjects were reported in 1943.¹² Although the material used at that time was in conjugated form, the fact was not emphasized in the report because distinction between the conjugated and free forms was not appreciated then. In this initial report, two patients had an established diagnosis of Addisonian anemia for some years before the experiment, while the others had chronic anemias due to various known and unknown causes. It was found during the study that untreated normal controls excreted from 1.5 to 3.5 micrograms of free B₁₂ in twenty-four hours, while determinations in patients with anemia (including pernicious anemia) prior to treatment showed total values as low as from 0.5 to 2.0 micrograms in twenty-four hour specimens of urine.

Further, in these early observations during the daily administration of from 2.8 to 4.2 mg. of B₁₂ conjugate (yeast concentrate) the excretion of free B₁₂ was found to be increased to values ranging from 94 to 334 micrograms in twenty-four hour specimens of urine. Subsequently, comparable values in urinary excretion were found in two additional patients with Addisonian anemia in severe relapse, who were treated with similar amounts of B₁₂ conjugate.

More recently, contrary observations on the utilization of Vitamin B₁₂ conjugate in pernicious anemia have been reported by Bethell, Swenseid, Bird, Meyers, Andrews and Brown⁵ and Welch, Heinle, Nelson and Nelson.²⁰ Bethell and his associates found free B₁₂ in the urine of five patients with pernicious anemia, during the daily oral administration of 4 mg. of B₁₂ conjugate, in amounts only equal to that present in the urine prior to treatment. They administered a conjugate prepared from yeast similar to that used in our above-mentioned experiments of 1943. It is also worthy of remark that there were no consequential hemopoietic phenomena observed by Bethell and his associates in their patients during the period of ten days' treatment.

Welch and his associates²⁰ gave B₁₂ conjugate (pteroylheptaglutamic acid) in a daily dose of 1 mg. orally to a patient with pernicious anemia in relapse for ten days without human gastric juice, and for eleven days with 100 cc. of human gastric juice during which time free Vitamin B₁₂ (pteroylglutamic acid) was not released. A second patient in relapse was given 0.85 mg. of free B₁₂ daily for ten days by the intramuscular route during which period there was a reticulocytosis of 3 per cent and an average urinary elimination of B₁₂ of 15 per cent. The same patient was then given 2.5 mg. of the B₁₂ conjugate daily by the intramuscular route for twelve days without hemopoietic response and with a urinary excretion not exceeding 1 per cent of free B₁₂. One large dose of 30 mg. of the

* Received for publication, July 2, 1947.

conjugate was also found to cause no hematologic changes, nor was any of the free form excreted in the urine during twelve days' observations.

In view of the contradictory observations on the oral utilization of B₁₂ conjugate by patients having established Addisonian anemia, a new series of four patients was selected by us for this study, two of whom were recently admitted, the other two being recidivists. All conformed to the clinical criteria of Addisonian anemia, showing the megaloblastic bone marrow characteristic of this disease. The Vitamin B₁₂ content of all urine specimens was determined by microbiologic assay as described by Bird, Bressler, Brown, Campbell and Emmett.⁶ The hematologic data, dosage and urinary excretion of Vitamin B₁₂ are enumerated in tabular form for each of the four patients of this series (see Tables 1 to 4).

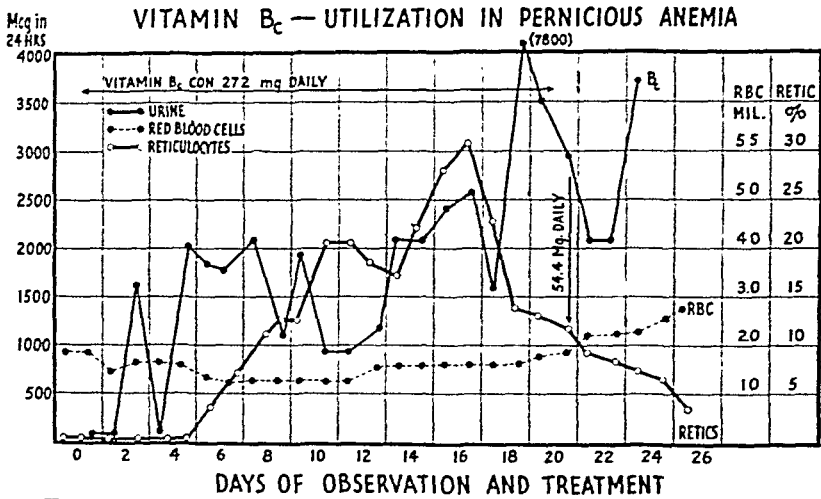


FIG. 1. The erythropoietic response and urine excretion following the daily oral administration of 27.2 mg. of Vitamin B₁₂ conjugate. Note the slight increase in erythrocytes after the conjugate was increased to 54.4 mg. daily.

THERAPEUTIC STATUS OF VITAMIN B₁₂

Since Pfiffner and his associates¹¹ announced the isolation and identification of a new member of the Vitamin B complex in 1943 as having "antianemia" value, aggressive investigations have been directed toward its evaluation in human anemias. Sharp, Vonder Heide and Wolter,¹² using yeast concentrates, demonstrated only minor hemopoietic improvement in pernicious anemia and in nonpernicious anemia, but as reported at that early date (1943), it was suspected that the dosage was suboptimal. Berry, Spies and Doan⁴ demonstrated an elevation of circulating leukocytes in some patients following intravenous injection of a Vitamin B₁₂ conjugate prepared from liver. The leukopoietic phenomena in these patients were due to an increase in neutrophils. Watson and co-workers¹⁹ studied the effect of *Lactobacillus casei* concentrates on refractory anemias, and particularly on leukopenia. In some patients who were made leukopenic by radiation, they observed a favorable leukopoietic effect but little change in the erythrocytes and in the hemoglobin. Moore, Bierbaum, Heinle and Welch¹⁰ extracted the vitamin from spinach and grass, but obtained

indifferent responses in pernicious and other macrocytic anemias. Castle and his associates⁷ fed purified casein, crystalline Vitamin B complex, and Vitamin B₁₂ in amounts varying from 2.3 to 3.6 mg. daily with negligible hemopoietic effect.

Little progress regarding the hemopoietic effect of Vitamin B₁₂ could be made because of its limited production from yeast and liver.

In 1945, Angier and associates¹ announced the synthesis of the *L. casei* factor or the free form of Vitamin B₁₂, which has been designated as folic acid. Innumerable clinical studies followed with the result, to date, that folic acid has been assessed as effective in macrocytic types of anemia and hemopoietically

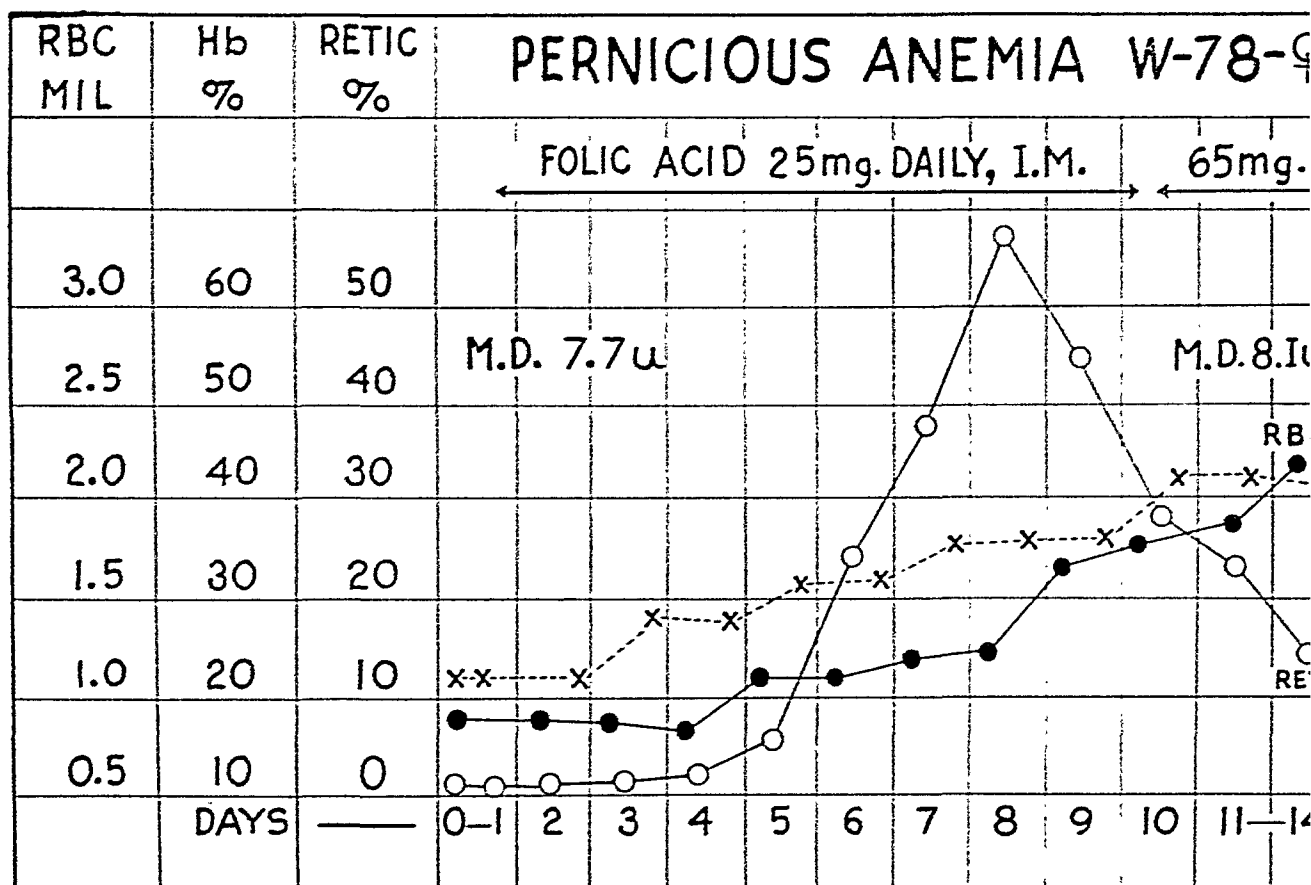


FIG 2. Response of a severely relapsed pernicious anemia to folic acid by the intramuscular route. The reticulocyte value of 58 per cent is approximately the ideal calculated per cent.

active in pernicious anemia. In the latter anemic state, however, the effective dose has been found to be variable by either oral or parenteral routes; daily amounts as small as 5 mg. have induced slow remission in some patients, while other patients have required from 50 to 100 mg. daily. The disturbing feature in treatment of Addisonian anemia with folic acid alone is the progressive neural changes that have been observed despite a favorable hemopoietic response. A report of two cases with this relapsing phenomenon was made in 1946 by Berman and Jacobson.² One of their patients showed progressive neurologic exacerbation despite the oral administration of 400 mg. of folic acid weekly, although the hemopoietic response on from 20 to 60 mg. daily followed the expected pattern of remission (see Fig. 2). Heinle and Welch⁸ observed

progressive neurologic relapse in pernicious anemia treated with folic acid, and suggested the possibility that it may actually precipitate exacerbation of pathologic neural processes. Meyer⁹ found that folic acid in doses of from 15 to 50 mg. orally and 20 mg. intramuscularly produced submaximal reticulocytosis in pernicious anemia, but failed to prevent the development of neurologic symptoms.

Steinkamp and co-workers¹⁷ and others have proved beyond doubt that folic acid is utilized in various forms of blood disorders, including anemia; the average excretion in normal states found by Steinkamp and associates, was about 2.34 micrograms in twenty-four hours. This figure for the normal person agrees with our own observation mentioned above.

UTILIZATION OF VITAMIN B₁₂ CONJUGATE

The nonutilization of Vitamin B₁₂ conjugate by patients having pernicious anemia, observed by several investigators cited above, cannot be explained readily in view of our original findings and the additional data in Tables 1 to 4. Three of the four patients in this series showed a high concentration of free Vitamin B₁₂ in the urine. The fourth patient (Table 4) showed a bizarre pattern of utilization in that appreciable quantities were excreted for two days following a daily dose of 6 mg. of the conjugate, and then the patient failed to excrete a calculable amount for three days. Following this negative phase, amounts of free B₁₂ were found in the urine comparable to normal excretion; then on the addition of Ventriculin, 30 Gm. daily, extremely large amounts of B₁₂ were present in twenty-four hour urine specimens. The hemopoietic effect throughout thirty days of observation was slight, although remission appeared to be impending after administration of Ventriculin was started.

Patient We (Table 1) showed a greater uniformity of utilization of B₁₂ conjugate. There were three determinations that did not conform to the general level, hence one would suspect error in the collection of urine samples. This patient showed a reticulocytosis of 22.4 per cent on the tenth day of treatment, and had a secondary rise to 30.8 per cent despite a pneumonic process. The highest urinary concentration of free B₁₂ was found during this period, 7800 micrograms in twenty-four hours, and was not equaled in the final ten days of treatment when the dose was doubled. Considered from a hematologic viewpoint, the prolonged reticulocytosis conformed to the pattern frequently seen when the dosage of potent antipernicious anemia factor is suboptimal. Further, infection would contribute to delayed erythropoiesis, hence it can be assumed that the amount of B₁₂ conjugate being given was inadequate, either because of suboptimal level for this patient, or because of a complicating infection. Ordinarily, however, if the dosage of potent antipernicious factor were optimal, satisfactory phenomena of early remission should have occurred within the nine day period prior to the onset of pneumonia.

Patient Hi (Table 2) was treated with the largest daily dose of B₁₂ conjugate, 54.4 mg., but showed the least hemopoietic effect and the slightest utilization. Doubtless these phenomena are correlatives, but the explanation is obscure.

TABLE 1
PATIENT WE
VITAMIN B₁₂ CONJUGATE IN PERNICIOUS ANEMIA

DATE, 1946		RBC MILLIONS	HEMOGLOBIN PER CENT	RETICULOCYTES PER CENT	LEUCOCYTES	URINE 24 HOUR EXCRETION B ₁₂ IN MICROGRAMS	ORAL DOSE, MG. B ₁₂ CONJUGATE
May	20	1.92	51	1.8	3,350		
	22					163.8	27.2
	23	1.65	42	0.8	3,400		(Equivalent to 10 mg. free folic acid)
	24	1.85	41	0.9	4,400	1667.5	27.2
	25			1.2		154.9	27.2
	26			1.8		2100	27.2
	27	1.50	36	4.5	9,800	1817	27.2
	28	1.35	32	7.5	9,400	1760	27.2
	29	1.44	38	10.0	15,650	2194.5	(Pneumonic signs)
	30			13.1		1000	27.2
June	31	1.43	34	14.4	15,150	1890	27.2
	1			22.4		931.7	27.2
	2			22.2			27.2
	3	1.62	39	17.8	15,200	1260	27.2
	4	1.77	39	16.2	13,000	2142	27.2
	5	1.74	43	24.2	11,000		27.2
	6	1.79	42	29.4	9,600	2400	27.2
	7	1.75	43	30.8	12,150	2640	27.2
	8			22.5		1680	27.2
	9			13.6		7800	27.2
	10	1.81	40	12.5	8,000	3640	27.2
	11	1.90	43	11.6	17,600	2860	54.4
	12	2.20	46	8.9	12,700	2100	54.4
	13	2.04	42	8.0	9,000	4.1	54.4
	14	2.04	50	7.9	8,400	4050	54.4
	18	2.40	47	6.8	21,650		

TABLE 2
PATIENT HI
VITAMIN B₁₂ CONJUGATE IN PERNICIOUS ANEMIA

DATE, 1946		RBC MILLIONS	HEMOGLOBIN PER CENT	RETICULOCYTES PER CENT	LEUCOCYTES	URINE 24 HOUR EXCRETION B ₁₂ IN MICROGRAMS	ORAL DOSE, MG. B ₁₂ CONJUGATE
June	6	1.06	32	1.8	2,200		
	7	1.17		1.2	7,500	1.0	54.4
	9	1.19		1.8			54.4
	10	1.26	34	1.7	4,200	3.9	54.4
	12	1.23	32	2.1	3,400	4.1	54.4
	13	1.30	31	2.3	4,150	3.5	54.4
	14	1.27	30	0.7		12.5	54.4
	15			3.0		6.6	54.4
	17	1.21		4.5		5.9	54.4
	19	1.27		9.2		1.5	54.4
	20			4.0		108.0	54.4
	21	1.27	33	6.6		170.0	54.4
	24	1.32	39	4.0			54.4
	25	1.40	36	5.7			54.4
	28	1.55	47	5.5	6,250		54.4
	3	1.75	54	2.8			54.4

It is well known, of course, that an occasional patient with Addisonian anemia fails to respond to administration of Ventriculin or liver when given orally in amounts even greater than the established daily dose. The possibility, therefore, is obvious that there may be the same unresponsiveness in the case of either conjugated or free Vitamin B₁₂ when given orally. This exception suggests that there are unknown factors involved in the metabolic processes of pernicious anemia. In this series of patients, the bone marrow showed the classic megaloblastic type, which would imply uniform responsiveness. It is suggested that exploration of other systems may be necessary before the difference between these types can be elaborated.

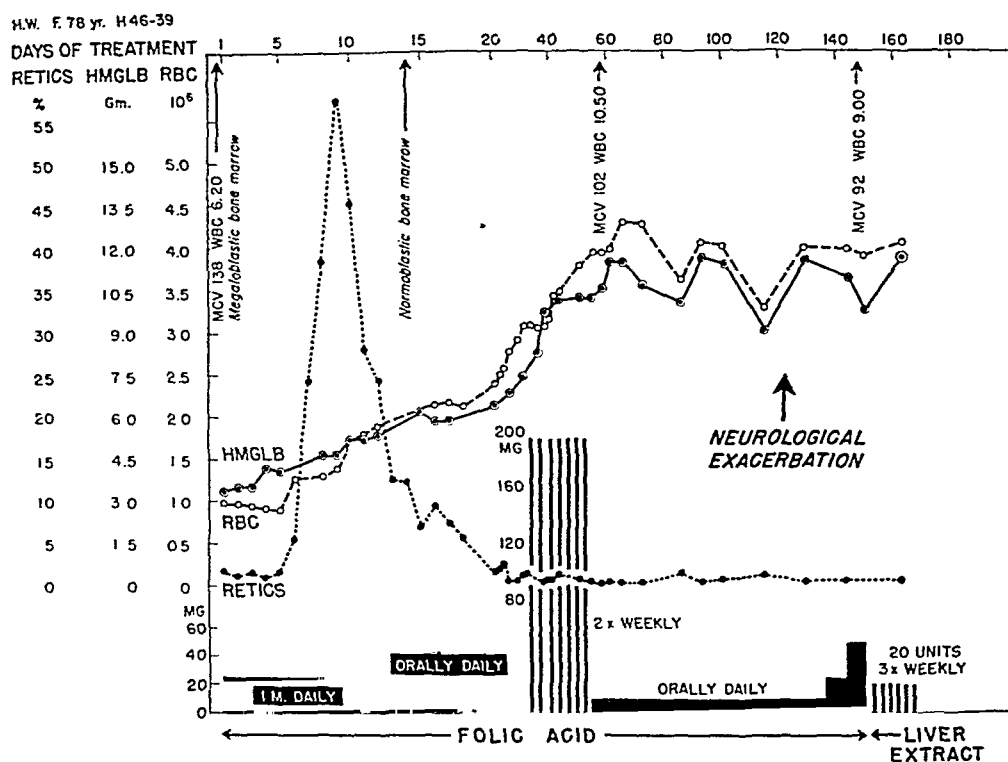


FIG. 3. Treatment of relapsed pernicious anemia with folic acid orally in a patient showing progressive severity in neural changes despite weekly dose of 400 mg.

Apparently the variability in utilization and in responsiveness of B₁₂ conjugate in pernicious anemia is not encountered with the free form in macrocytic anemia. Spies,¹³ Berry and Spies,³ Spies, Vilter, Koch and Caldwell¹⁶ have found folic acid effective in macrocytic anemia of various types and Spies, Lopez, Menendez, Minnich and Koch¹⁴ have also demonstrated the hemopoietic efficiency of the vitamin in sprue. More noteworthy and pertinent to this particular study is the reported effectiveness of Vitamin B₁₂ conjugate in the treatment of tropical sprue by Suarez, Welch, Heinle and Suarez.¹⁸ Further, Spies, Lopez, Milanes and Aramburu¹⁵ have confirmed the observations of Suarez and associates regarding the therapeutic efficacy of folic acid, not only in sprue, but in relapsed Addisonian anemia and nutritional macrocytic anemia, significantly enough by the use of a *synthetic conjugate of folic acid*.

TABLE 3
PATIENT Mo
VITAMIN B₁₂ CONJUGATE IN PERNICIOUS ANEMIA

DATE, 1946	RBC MILLIONS	HEMOGLOBIN PER CENT	RETICULOCYTES PER CENT	LEUKOCYTES	URINE 24 HOUR EXCRETION B ₁₂ IN MICROGRAMS	ORAL DOSE, MG. B ₁₂ CONJUGATE
Sept. 10	1.95	50	1.0	2,700		
11	1.97	51	0.4	4,100		
12	2.05	49	0.6	2,600		
13	2.04	55	0.2	3,650		
14			1.5		6.11	
15			0.7		15.8	27.2
16	2.14	48	0.8	3,350	6.05	27.2
17	2.12	52	0.5	3,400	121.04	27.2
18	2.15	47	1.0	3,000	796.16	27.2
19	2.10	43	0.9	3,600	1430.00	27.2
20	1.90	42	2.6	2,900	489.90	27.2
21			1.0		220.8	27.2
22			4.0		650.00	27.2
23	2.29	48	4.3	2,800	492.00	27.2
24	2.20	50	6.9	5,000	1380.00	27.2
25	2.26	52	6.4	3,850	1966.50	27.2
26			3.0	4,900	78.00	27.2
27	2.45	51	2.3	6,200	735	27.2
28			3.8		26.0	Discontinued
29			4.6		11.25	
30	2.40	51	2.2	7,400		

TABLE 4
PATIENT Fe
VITAMIN B₁₂ CONJUGATE IN PERNICIOUS ANEMIA

DATE, 1946	RBC MILLIONS	HEMOGLOBIN PER CENT	RETICULO- CYTES PER CENT	LEUKOCYTES	URINE 24 HOUR EXCRETION B ₁₂ IN MICROGRAMS	MEDICATION
May 22	3.08	71	1.0	6,000	1.9	B ₁₂ conjugate 6 mg. daily orally.
23	3.00	65	0.9	4,250	756.0	
24	3.16		0.8	4,500	787.2	
27	2.84	74	0.9		67.8	
29	2.76	74	0.4	4,700	17.5	
June 2	3.03	69	1.0	5,100		Ventriculin with iron 30 gm. ÷ B ₁₂ 6 mg. daily
4	3.14	76	0.6	6,950		
5	2.85	72	0.9	5,500	3.3	
9	2.80	64	0.8	7,600	3.1	
14	2.86	70	0.8	6,400	3.4	
17	2.95	71	1.0	4,550	3000	
19	3.26	76	1.7	5,500	3045	
21	3.35	81	1.5	5,900	4960	
24					1432	
25	3.25	76	1.0	5,900		

SUMMARY

Controversy has existed as to the utilization of Vitamin B₁₂ conjugate by patients in the relapsed phase of pernicious anemia. While the scarcity of this form of the vitamin in pure state has limited the observations to a few cases only, the data cited herein confirm our less exact observation reported previously: that excretion in the urine of the free form of B₁₂ in high concentrations follows the oral administration of the conjugate in doses ranging from 6 to 27.2 mg.

In contrast to the above positive values after the oral use of the B₁₂ conjugate, one patient given 54.4 mg. daily failed to show any appreciable utilization as judged by urinary excretion or by hemopoietic changes. A second patient also failed to excrete the vitamin until Ventriculin was given. As was true with our patients previously treated, the present series of patients in relapsed pernicious anemia did not manifest any striking hemopoietic responses other than reticulocytosis in several instances. It is assumed that the dosage was suboptimal, or that complications prevented the usual hematologic response.

The vitamin in the form of synthetic folic acid induced a satisfactory erythropoietic response in severely relapsed Addisonian anemia but failed to prevent progressive neurologic changes in some instances.

Since Spies and his associates have demonstrated hemopoietic efficacy in pernicious anemia and other macrocytic anemias by the use of a synthetic folic acid in the conjugated form, it is believed that the probable negative results with the natural conjugate, as reviewed in this report, are due to suboptimal dosage and possibly, to some degree, are attributable to an individual variability of metabolism in Addison's anemia when this substance is given by the oral route.

Acknowledgment. The authors acknowledge with thanks the cooperation of Dr. O. D. Bird, Head of the Chemical Vitamin Research Division of Parke, Davis and Company, in making the determinations of Vitamin B₁₂ in all urine specimens.

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EFFECT OF LIPIDS ON KAHN ANTIGEN.

III. INCREASING SENSITIVITY OF KAHN STANDARD ANTIGEN TO THE LEVEL OF KAHN SENSITIZED ANTIGEN BY ADDITION OF ALCOHOLIC EXTRACT OF SOY BEAN LECITHIN*

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In an earlier publication² it was reported that the addition of a purified alcoholic extract of soy bean lecithin to undersensitive Kahn antigen increased its titer with salt solution and its sensitivity with syphilitic serums. Subsequent experiments with this method indicated that a simple alcoholic extract of commercial soy bean lecithin could also be used successfully for this purpose. On the basis of this latter observation, attempts were undertaken to employ such an extract in the preparation of antigen possessing the titer and sensitivity requirements of Kahn sensitized antigen. The results summarized below indicate that sensitized antigen can be prepared by the addition of an alcoholic extract of commercial soy bean lecithin to Kahn standard antigen.

MATERIALS AND METHODS

Antigens. Four standard antigens were selected for this study: (1) Lot 92 which had been corrected by the addition of a small amount of sensitizing reagent and by dilution with 0.6 per cent cholesterolized alcohol; (2) Lot 97 which had been corrected by dilution with cholesterolized alcohol; (3) Lot 98 which had been corrected by the addition of egg lecithin and (4) Lot 99 which had been corrected by dilution with cholesterolized alcohol. Antigens Lot 92 and Lot 97 were prepared from single lots of Bacto-Kahn beef heart while Lot 98 and Lot 99 represented mixtures prepared from two or three lots of the beef heart.

Soy bean lecithin. A solution of lecithin was prepared by adding 1 gm. of commercial soy bean lecithin (furnished by the Difco Laboratories) to 100 ml. of 0.6 per cent cholesterolized absolute alcohol. This solution was placed in a water bath at 56 C. for about thirty minutes, allowed to stand over-night at room temperature and then filtered. Two solutions were made in this way from two lots of commercial soy bean lecithin, No. 14662 and No. 16597 (Difco). The former was more highly oxidized than the latter.

Method of antigen titration. This technic was the same as that described for Kahn standard antigen,¹ except that larger amounts of 0.9 per cent sodium chloride solution were used in preparing the antigen:salt solution mixtures. Generally, five suspensions of an antigen were prepared by mixing 1 ml. quantities of antigen with 1.9, 2.0, 2.1, 2.2 and 2.3 ml. of 0.9 per cent sodium chloride solution. A control was set up at the same time using a known lot of

* Aided by a grant from the Difco Laboratories, Inc., Detroit, Michigan. Received for publication, June 23, 1947.

Kahn sensitized antigen at its indicated titer. Three-tube Kahn tests were employed to determine the antigen titer, using 0.9 per cent salt solution instead of serum. The description of the titration readings ranging from heavy non-dispersible aggregates to those of water clarity are listed below, together with abbreviated designations when abbreviations are used.

Heavy aggregates—Heavy agg.

Cloudy, aggregates—Cloudy, agg.

Cloudy, fuzzy

Cloudy

Slightly cloudy—Sl. cloudy

Trace cloudy—Tr. cloudy

Opalescent (Titer)

Trace clear—Tr. clear

Slightly clear—Sl. clear

Slightly too clear—Sl. too clear

Too clear

Watery clear

Methods of converting Kahn standard antigen to sensitized antigen. Ten ml. samples of two of the standard antigens, Lot 97 and Lot 98, were diluted 5, 10 and 20 per cent with a 1 per cent soy bean lecithin solution. These samples were titrated with 0.9 per cent sodium chloride solution, according to the above procedure. The samples diluted 10 and 20 per cent with soy bean lecithin solution gave very cloudy and unsatisfactory titration pictures. Hence, samples of the other two antigens, Lot 92 and Lot 99, were only diluted 5 per cent with this lecithin solution to obtain a preliminary titration picture.

After an acceptable titer had been obtained, the sensitivities of the antigens were tested with syphilitic and nonsyphilitic serums, using Kahn sensitized antigen as a control. Further adjustments were made when necessary, by decreasing the amount of soy bean lecithin added to the antigen, by the addition of cholesterolized absolute alcohol and/or by small adjustments in the amount of salt solution used in the titer. A 50 ml. sample of each antigen was then prepared according to the corrections determined for the 10 ml. test sample and was rechecked by making comparative tests with a control lot of Kahn sensitized antigen.

EXPERIMENTAL

1. *The effect of an alcoholic extract of soy bean lecithin on the titration picture of Kahn standard antigen.* Two lots of Kahn standard antigen, 97 and 98, were diluted as indicated, 5, 10 and 20 per cent with a one per cent solution of soy bean lecithin No. 16597. Titrations were performed on the diluted antigens employing 0.9 per cent sodium chloride solution. The results of this experiment are given in Table 1.

It can be seen from Table 1 that 5 per cent dilution with the soy bean lecithin solution increased the titer of the two antigens used from the standard to the sensitized range. Thus, while the titer of Lot 97 and Lot 98 was $1 \div 1.3$, a

5 per cent dilution of the antigens with soy bean lecithin solution raised the titer to about $1 + 2.2$. However, 10 and 20 per cent dilutions with this lecithin solution produced cloudiness and aggregates throughout the titration pictures and no titer was obtained.

2. *The effect of alcoholic extracts of different lots of soy bean lecithin on the titration picture of Kahn standard antigen.* The first experiment showing the capacity of soy bean lecithin to increase the titer of Kahn standard antigen was made with a 1 per cent lecithin solution No. 16597. Another 1 per cent solution was prepared from soy bean lecithin No. 14662, a more highly oxidized material which produced a dark amber colored extract. A study was undertaken to

TABLE 1
CHANGES IN TITRATION PICTURE OF KAHN STANDARD ANTIGEN FOLLOWING THE ADDITION OF ALCOHOLIC EXTRACTS OF SOY BEAN LECITHIN

TITRATION PICTURE OF KAHN STANDARD ANTIGEN		TITRATION PICTURE OF ANTIGENS DILUTED WITH SOY BEAN LECITHIN			
Amounts in ml. of anti- gen and salt solution	Titration readings	Amounts in ml. of anti- gen and salt solution	Titration readings of antigens diluted with various amounts of a 1% solution of soy bean lecithin extract		
			5% dilution	10% dilution	20% dilution
Antigen Lot 97					
1 + 1.1	Tr. cloudy	1 + 1.9	Sl. cloudy	Sl. cloudy	Cloudy
1 + 1.3	Opalescent*	1 + 2.0	Tr. cloudy	Tr. cloudy	Cloudy, agg.
1 + 1.5	Tr. clear	1 + 2.1	Tr. cloudy	Tr. cloudy	Cloudy, agg.
1 + 1.7	Sl. clear	1 + 2.2	Opalescent	Tr. cloudy	Sl. cloudy
Antigen Lot 98					
1 + 1.1	Sl. cloudy	1 + 1.9	Cloudy, agg.	Cloudy, agg.	Heavy agg.
1 + 1.3	Opalescent	1 + 2.0	Cloudy, agg.	Cloudy, agg.	Heavy agg.
1 + 1.5	Tr. clear	1 + 2.1	Sl. cloudy, agg.	Cloudy, agg.	Heavy agg.
1 + 1.7	Sl. too clear	1 + 2.2	Sl. clear	Sl. cloudy	Sl. cloudy

* Opalescent indicates the titer. No titer was obtained in those titrations which do not show opalescence at some point.

compare the effect of these two extracts on the titer and titration picture of Kahn standard antigen. The results of this study are given in Table 2.

It is shown in Table 2 that the lecithin solution prepared from the more oxidized material, No. 14662, is the more active. It can be seen that dilutions of both antigens with this soy bean lecithin solution showed higher titers than similar dilutions made with lecithin solution No. 16597. This finding suggests that the degree of oxidation of the gummy lecithin material may affect the characteristics of the extract made from it.

3. *The sensitivity and specificity adjustments of Kahn standard antigens raised to the level of sensitized antigen by alcoholic extracts of soy bean lecithin.* The titration pictures resulting from the addition of soy bean lecithin, as listed in Tables 1 and 2, indicated that about a 5 per cent dilution of the antigen with a

stock 1 per cent lecithin solution was sufficient to raise the sensitivity level of Kahn standard antigen to that of Kahn sensitized antigen. Thus, an arbitrary 5 per cent dilution was made with each of the four lots of Kahn standard antigen used in this study. After the preliminary titrations had been carried out, each of the antigens to which soy bean lecithin extract solution had been added was tested with weakly positive syphilitic serums and the results compared with those obtained with Kahn sensitized antigen. On the basis of these comparative tests, final adjustments for sensitivity were made by the addition of cholesterolized alcohol or by small changes in the titer. An illustration of the correction of one lot, 92, is presented in Table 3.

TABLE 2

EFFECT OF ALCOHOLIC EXTRACTS OF TWO DIFFERENT LOTS OF SOY BEAN LECITHIN ON THE TITRATION PICTURE OF KAHN STANDARD ANTIGEN

TITRATION PICTURE OF KAHN STANDARD ANTIGEN		TITRATION PICTURE OF ANTIGENS DILUTED WITH 1% SOY BEAN LECITHIN			
Amounts in ml. of antigen and salt solution	Titration readings	Amounts in ml. of antigen and salt solution	Dilution with soy bean lecithin solution	Lot No. 14662	Lot No. 16597
				Titration readings	
Antigen Lot 97					
1 + 1.1	Tr. cloudy	1 + 2.0	5%	Cloudy	Tr. cloudy
1 + 1.3	Opalescent*	1 + 2.1		Sl. cloudy	Tr. cloudy
1 + 1.5	Tr. clear	1 + 2.2		Sl. cloudy	Opalescent
1 + 1.7	Sl. clear	1 + 2.3		Tr. cloudy	Tr. clear
		1 + 2.4		Tr. cloudy	Tr. clear
Antigen Lot 99					
1 + 1.1	Tr. cloudy	1 + 2.0	4%	Tr. cloudy	Tr. cloudy
1 + 1.3	Opalescent	1 + 2.1		Tr. cloudy	Opalescent
1 + 1.5	Tr. clear	1 + 2.2		Tr. cloudy	Tr. clear
1 + 1.7	Sl. clear	1 + 2.3		Opalescent	Tr. clear

* Opalescent indicates the titer.

Table 3A shows that Kahn standard antigen Lot 92 diluted 5 per cent with soy bean lecithin gives a satisfactory titration picture, indicating that 1+2.1 is the titer. As seen in Table 3B, however, the reactions with syphilitic serums at that titer were not as sensitive as those with the Kahn sensitized antigen control. When the tests were repeated employing a titer of 1 + 2.0, the reactions were slightly more sensitive than the control tests. Thus, a titer of 1 + 2.05 was used in a third set of tests (serums 11 to 15 in the table) and in this case the sensitivity results were comparable to Kahn sensitized antigen and the opalescence of the negative reactions was satisfactory.

Ten ml. samples of each of the four antigens were adjusted to the level of Kahn sensitized antigen in this way. The final methods of correction are given in Table 4.

Antigen Lot 92 was diluted 5 per cent with a 1 per cent solution of soy bean lecithin solution No. 16597 and was used at a titer of $1 + 2.05$. Antigen

TABLE 3

ADJUSTMENT OF SENSITIVITY OF KAHN STANDARD ANTIGEN TO THE LEVEL OF SENSITIZED ANTIGEN BY THE ADDITION OF ALCOHOLIC EXTRACT OF SOY BEAN LECITHIN.

A. PRELIMINARY TITRATION FOR DETERMINATION OF TITER

AMOUNTS IN ML. OF ANTIGEN AND SALT SOLUTION	TITRATION READINGS OF KAHN STANDARD ANTIGEN LOT 92 DILUTED 5% WITH A 1% SOY BEAN LECITHIN SOLUTION
$1 + 1.9$	Trace cloudy
$1 + 2.0$	Trace cloudy
$1 + 2.1$	Opalescent*
$1 + 2.2$	Trace clear
$1 + 2.3$	Slightly clear

* Opalescent indicates the titer.

B. ADJUSTMENT OF TITER ACCORDING TO ANTIGEN SENSITIVITY WITH SYPHILITIC SERUMS

SERUM	ANTIGEN LOT 92, 5% DILUTION WITH 1% SOY BEAN LECITHIN SOLUTION	SENSITIZED ANTIGEN (CONTROL)
	Titer $1 + 2.1$	Titer $1 + 2.35$
1	— — —	— — —
2	— \pm 1	\pm 1 1
3	— — —	— — 1
4	— — $\frac{1}{2}$	— \pm $\frac{1}{2}$
5	— — —	— \pm 2
	Tr. clear*	
	Titer $1 + 2.0$	
6	— — —	— — —
7	— \pm $\frac{1}{2}$	— \pm $\frac{1}{2}$
8	— \pm $\frac{1}{2}$	— \pm 3
9	— 1 $\frac{1}{2}$	— \pm $\frac{1}{2}$
10	\pm $\frac{1}{2}$ $\frac{1}{2}$	\pm 3 $\frac{1}{2}$
	Tr. cloudy*	
	Titer $1 + 2.05$	
11	— — —	— — —
12	— \pm $\frac{1}{2}$	— \pm $\frac{1}{2}$
13	— \pm 3	— \pm 3
14	— \pm $\frac{1}{2}$	— \pm $\frac{1}{2}$
15	\pm 3 $\frac{1}{2}$	\pm 3 $\frac{1}{2}$
	Opalescent*	

* Appearance of negative reactions.

Lot 97 was diluted similarly and used at a titer of $1 + 2.15$. This latter antigen was also corrected by a 5 per cent dilution with the 1 per cent soy bean lecithin solution No. 14662 and used at a titer of $1 + 2.4$. Thus indicating, as mentioned

above, that solution No. 14662 is the more active. Antigen Lot 98 was corrected by 5 per cent dilution with 1 per cent soy bean lecithin solution No. 16597 plus 20 per cent dilution with 0.6 per cent cholesterolized absolute alcohol and used at a titer of $1 \div 2.2$. Antigen Lot 99 was corrected by 4 per cent dilution with solution No. 16597 and also by 4 per cent dilution with 1 per cent solution No. 14662. The former was used at a titer of $1 \div 2.15$ and the latter at a titer of $1 \div 2.2$.

4. *The comparative results and keeping qualities of larger amounts of corrected antigens.* Fifty ml. samples of each antigen were prepared according to the correction determined by the 10 ml. test samples. These larger amounts were titrated and then compared with Kahn sensitized antigen using syphilitic and nonsyphilitic serums. The sensitivity of the 50 ml. samples compared closely with that of Kahn sensitized antigen and only small adjustments in the titers were necessary. These same samples were rechecked after about three months of

TABLE 4
METHODS FOR PREPARATION OF 10 ML. AND 50 ML. SAMPLES OF SENSITIZED ANTIGEN AND A COMPARISON OF THE TITERS OBTAINED

KAHN STANDARD ANTIGEN	CONVERSION TO SENSITIZED ANTIGEN BY DILUTION WITH:	TITER OF 10 ML. SAMPLES	TITER OF 50 ML. SAMPLES
Lot 92	5% lecithin No. 16597*	$1 \div 2.05$	$1 \div 2.0$
Lot 97	5% lecithin No. 16597	$1 \div 2.15$	$1 \div 2.25$
Lot 97	5% lecithin No. 14662	$1 \div 2.4$	$1 \div 2.5$
Lot 98	5% lecithin No. 16597 and 20% cholesterolized alcohol	$1 \div 2.2$	$1 \div 2.15$
Lot 99	4% lecithin No. 16597	$1 \div 2.15$	$1 \div 2.15^\dagger$
Lot 99	4% lecithin No. 14662	$1 \div 2.2$	$1 \div 2.3$

* Lecithin solutions No. 16597 and No. 14662 were both one per cent alcoholic extracts of soy bean lecithin.

† Two 4 liter lots were prepared in this same manner and each had a titer of $1 \div 2.15$ also.

storage at room temperature and they were found to have the same titers and sensitivity as when originally tested.

Two four liter amounts of standard antigen Lot 99 were adjusted to the level of sensitized antigen by diluting 4 per cent with 1 per cent soy bean lecithin solution No. 16597, and the titer was found to be the same as that of the 50 ml. sample. These antigens were tested with weakly positive syphilitic serums and gave results comparable to those obtained with Kahn sensitized antigen.

SUMMARY

A method for increasing the sensitivity level of Kahn standard antigen to that of Kahn sensitized antigen by the addition of an alcoholic extract of commercial soy bean lecithin is described. Six sensitized antigens were prepared from four lots of Kahn standard antigen by employing two lots of soy bean lecithin in different combination. Each newly prepared sensitized antigen showed the

same sensitivity with weakly positive syphilitic serums as the Kahn sensitized antigen control. When the sensitized antigens were rechecked after three months of storage at room temperature, they showed no appreciable changes in titer and sensitivity.

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RECOVERY OF GROUP O RECIPIENT AFTER TRANSFUSION OF TWO LITERS OF GROUP A BLOOD.

REPORT OF CASE WITH AUTOPSY FOLLOWING ULTIMATE DEATH FROM CARCINOMA OF LUNG AND PYOTHORAX*

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The reported mortality from transfusion of blood incompatible with respect to the major blood groups is approximately 50 per cent² and is roughly proportional to the quantity of blood given.^{1, 2, 6, 12} Death occurred within twenty-four hours in most cases when more than 500 cc. of incompatible blood was transfused. In the case reported in this paper, 2000 cc. of group A blood was transfused into a group O patient as a result of improper identification of the recipient when blood was drawn for cross matching. The patient apparently recovered completely from the hemolytic reaction but died fifty-three days later of carcinoma of the lung and pyothorax. Autopsy failed to reveal more than minimal evidence of renal damage or other abnormalities which could reasonably be attributed to the transfusion.

REPORT OF CASE

The patient, a 58 year old white man, was admitted to the Strong Memorial Hospital on November 16, 1946, complaining of cough and pain in the chest. A diagnosis of epidermoid carcinoma of the right lung was made on biopsy and an abscess on the right side of the chest was drained on December 3. On admission, the laboratory findings were as follows: erythrocyte count, 3,730,000; hemoglobin, 11.8 gm. per cent; leukocyte count, 13,500; nonprotein nitrogen, 44 mg. per cent; total protein, 5.1 gm. per cent; chlorides, 482 mg. per cent and carbon dioxide combining power 59 volumes per cent.

The patient was given 92 gm. of sulfadiazine orally between November 17 and December 3, 7,250,000 units of penicillin in saline intramuscularly between November 17 and December 30, and 1,300,000 units of aerosol penicillin between November 26 and December 3. On December 4, a right pneumonectomy was performed under intratracheal nitrous oxide, oxygen and ether anesthesia. It was found that an epidermoid carcinoma involved approximately three-fourths of the upper lobe of the lung and that there was extensive necrosis of the tumor mass. A transfusion of 2000 cc. of group A blood from four donors was started in the operating room and was finished eight hours later in the

* The laboratory studies described in this report were carried out in part in connection with a contract between the University of Rochester (Department of Medicine, School of Medicine and Dentistry) and the Office of Naval Research. Received for publication, July 25, 1947.

recovery room before the patient had regained consciousness; 1000 cc. of normal saline was also given intravenously during the operation. The operative and immediate postoperative courses were not remarkable. There had been an irregular fever with peaks as high as 39.8 C. prior to operation, but on December 4, the maximum temperature was only 38.2 C., and for the next sixteen days the temperature did not rise above 38.0 C. On the day following operation, jaundice and dark urine were noted, but there was no evidence of peripheral vascular collapse and the patient had no specific complaints. He repeatedly denied having any pain in the back.

Investigation^{8, 15} on December 5, the day following transfusion and operation, revealed that the patient's blood was recorded as group O on a previous hospital admission for bleeding duodenal ulcer in 1942, at which time 1000 cc. of group O blood was transfused without reaction. The patient's pre-transfusion specimen of blood received at the blood bank on December 3, was re-examined and was again found to be group A. Repetition of the cross match of this specimen with each of the four donors showed no agglutination.

The patient's (undiluted) serum, drawn twenty-four hours *after* transfusion, however, produced strong but incomplete (3 plus) agglutination of the cells of three A₁ donors, and weaker (2 plus) agglutination of the cells of the fourth donor whose subgroup was A₂. The cells in the patient's circulation twenty-four hours after transfusion were only very slightly agglutinated by anti-A serum, a finding which indicated that nearly all of the donated cells had already been destroyed. Further investigation revealed that, due to mistaken identity, the pre-transfusion specimen had been drawn from another patient.

Two days after transfusion, the patient's total serum bilirubin concentration was 11.58 mg. per cent; the direct one-minute fraction⁵ was 5.38 mg. per cent. At this time the icteric index was 63 and the hemoglobin concentration in the serum was 269 mg. per cent. Spectroscopic studies confirmed the presence of hemoglobin and showed no absorption bands indicative of methemoglobin or methemalbumin.

The urine on the day following transfusion of incompatible blood was dark in color and contained a few red blood cells. The presence of bilirubin was demonstrated by a 2 plus Harrison test¹¹ and positive foam and methylene blue tests. The guaiac test was strongly positive. Spectroscopic examination of the urine confirmed the presence of hemoglobin. The urine became normal in color on the fourth day, but a trace of albumin was present until the sixteenth day after transfusion and intermittently thereafter.

The red blood cell count dropped to 2,640,000 and the hemoglobin to 9.5 gm. per cent during the first forty-eight hours after transfusion of the incompatible blood (Fig. 1). A leukocytosis of 24,000 developed postoperatively and continued at this level for nearly a month. The nonprotein nitrogen rose to 100 mg. per cent three days after transfusion and reached a maximum of 166 mg. per cent six days after transfusion, then slowly fell to normal in the course of the next month (Fig. 1). The creatinine rose to a maximum of 8.3 mg. per cent six days after transfusion and fell to 3.5 mg. per cent in three weeks. The icteric index decreased rapidly from 63 to 14 in six days (Fig. 1).

The isoagglutinin titers against A₁, A₂ and B cells are also shown in Figure 1. In carrying out these titrations the method of Wiener^{13a} was employed with the exception that results were expressed in terms of *final* dilutions of serums after

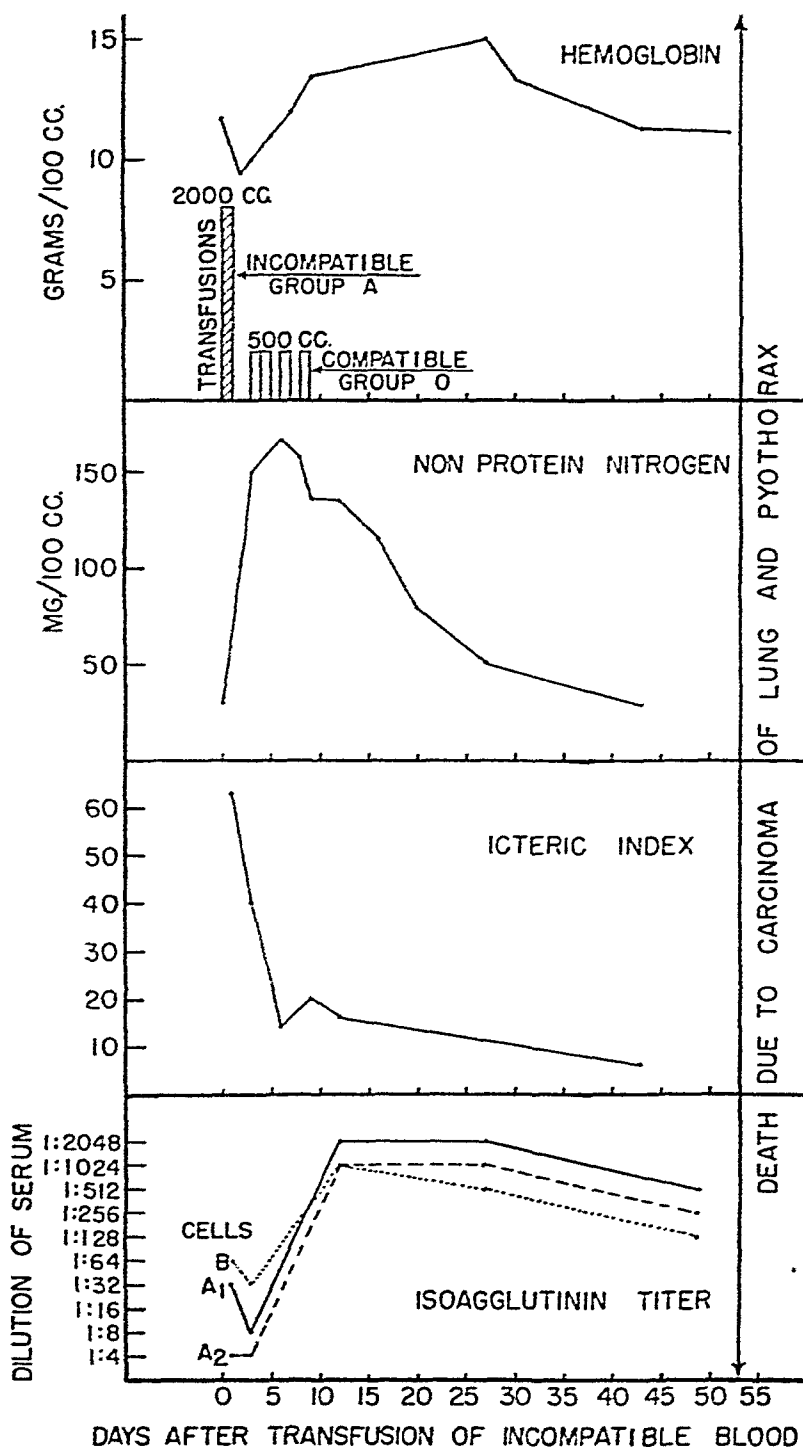


FIG. 1

addition of cell suspensions. Titrations were also carried out with 25 per cent human albumin solution as a diluent (instead of saline) for both serums and cell suspensions. In nearly every case titers were lower when albumin was

thus employed. The titer of isoagglutinins prior to transfusion could not be determined because no pre-transfusion specimens of the patient's blood were available. The low titers against A_1 and A_2 cells immediately after transfusion may be attributed to absorption by the donated cells, while the sharp subsequent rise in titer against these cells is no more than might be expected after massive antigenic stimulation.^{4, 13b} The rise in anti-B titer in a group O recipient after transfusion of A blood is more difficult to explain and is contrary to Wiener's^{13b} experience.

When it was discovered twenty-four hours after transfusion that incompatible blood had been given, fluids and alkali were administered liberally. One liter of one-sixth molar sodium lactate was given subcutaneously and 4 gm. of sodium bicarbonate orally, followed by 2 gm. of sodium bicarbonate every four hours for the next four days. In addition, the patient received 500 cc. of 10 per cent dextrose in distilled water on December 6 and 7, and 500 cc. of compatible group O whole blood on December 8, 9, 11 and 13. The recorded volumes of fluid intake on December 4, 5, 6 and 7 were 3000, 4400, 3350 and 2700 cc., respectively, and the urine volumes on these days were 325, 375, 525 and 700 cc., respectively. The urine volumes on subsequent days were considerably larger. An alkaline urine was maintained throughout the first week following transfusion, but unfortunately the patient's urine was not examined on the day of transfusion and hence the pH of the urine at that time is not known.

Although the patient made an apparently complete clinical recovery from the hemolytic transfusion reaction, his general condition began to grow worse about three weeks following operation. He developed remittent fever and anorexia, became progressively weaker and died on January 26, 1947, fifty-three days after operation. Autopsy revealed metastatic carcinoma of the pleura, mediastinal lymph nodes and liver. Microscopic examination of the kidneys revealed cloudy swelling and a moderate amount of old scarring. A few scattered casts, apparently containing hemoglobin pigment, and the presence of iron staining granules in interstitial macrophages and in the epithelium of some tubules, were the only residual evidence of previous hemoglobinemia.

DISCUSSION

To the best of our knowledge there are no reports in the literature of apparent clinical recovery following transfusion of incompatible (intergroup) blood in amounts as large as 2000 cc. This case is therefore considered of especial interest despite the fact that measurements of the degree of hemoglobinemia, methemalbuminemia, hemoglobinuria and of the urinary pH were not made during the crucial twenty-four-hour period immediately following transfusion.

A number of questions are raised by the observations made on this patient, but unfortunately few, if any, of these questions can be answered with certainty in the light of available information.

1. What mechanism protected the kidneys to the extent that fatal anuria did not develop, despite the fact that approximately 5 gm. of hemoglobin per Kg.

of body weight was liberated by destruction of the incompatible blood during the first twenty-four hours after transfusion? Did the condition of the kidneys (diminished filtration?) during the critical period of hemoglobinemia prevent the escape of hemoglobin into the tubules in quantities large enough to cause severe renal blockage? It is possible that the urine was alkaline at the time of transfusion and that this was a protective factor. Present controversies,^{7, 13, 16} however, leave room for doubt as to the efficacy of the alkali administered during the second to sixth days after transfusion.

2. Was the reaction relatively mild because of the patient's low titer of anti-A agglutinins? As previously explained, the titer of isoagglutinins in this case at the time of transfusion is unknown, and the low anti-A titers of the serums drawn soon after transfusion may be attributed to absorption of agglutinins by the donated cells. On the other hand, Davidsohn³ has reported low isoagglutinin titers in serums from patients with leukemia, and one of the authors (L.E.Y.) has found low titers in serums from patients with multiple myeloma and carcinomatosis. Wiener and Schaefer,¹⁴ moreover, have reported a case in which a leukemic patient of group O received 1500 cc. of group B blood without developing symptoms, despite the fact that the donated cells were eliminated within a period of twelve days. It is reasonable to believe that the severity of hemolytic reactions may depend to a considerable extent upon the recipient's titer of isoagglutinins against the transfused cells and that some patients with malignancy may, therefore, be expected to have relatively mild reactions. Operation of such a factor in this case, however, is perhaps rendered somewhat unlikely by the finding of an anti-A₁ titer of 1:32 and an anti-B titer of 1:64 twenty-four hours after transfusion. Since anti-A titers are usually higher than anti-B titers in group O serums, it seems possible that the patient's anti-A titer prior to transfusion might have been as high as 1:64 or 1:128.

3. Was the outcome of the reaction greatly modified by anesthesia? It is well known that anesthesia masks the signs and symptoms of hemolytic transfusion reactions.⁹ Information is lacking, however, on the effect of anesthesia on the pathologic physiology and mortality rates following transfusion of incompatible blood.

SUMMARY AND CONCLUSIONS

A case is reported in which 2000 cc. of group A blood was transfused into an anesthetized group O patient, who recovered from the hemolytic reaction, but died fifty-three days later of carcinoma of the lung and pyothorax. This unusual case serves to emphasize the following points with regard to hemolytic transfusion reactions:

1. The pathologic physiology of such reactions is by no means clear. The recipient's chances of survival may depend not only on the quantity of blood transfused, but also on such factors as urinary pH, isoagglutinin titer, presence of malignancy and infection and state of anesthesia.

2. Hemolytic reactions occurring in patients under general anesthesia may not be detected unless close attention is paid to the development of jaundice, oliguria and hemoglobinuria.

3. When blood grouping and matching are repeated following a suspected hemolytic reaction, it is essential that examination be made of the patient's blood taken *after* transfusion as well as of the patient's blood taken before transfusion.

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DIFFUSE PRIMARY ALVEOLAR CARCINOMA OF THE LUNG

(SO-CALLED "ALVEOLAR CELL" TUMOR OF LUNG)*

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In recent years numerous reports have been published of a unique type of primary lung tumor occurring in man. These tumors are characterized by an unusual type of nonciliated, tall, columnar and questionably mucus-secreting cells which line and are loosely attached to apparently unaltered alveolar walls, show papillary formation within alveoli and frequently desquamate into the alveoli. The tumors occur almost invariably in both lungs. About one-half of the tumors are found localized to the lungs at the time of death, often without evidence of lymphatic invasion, and the remainder show either local or distant metastases. None of the tumors shows the ordinarily accepted and classic features of primary bronchiogenic carcinoma, such as invasion of bronchial wall with or without stenosis or ulceration and/or atelectasis. Similarly, no primary tumor in any other situation is found so that metastatic tumor can be definitely ruled out. Finally, in almost all instances, the point of origin, whether from bronchiolar basilar epithelium, infundibular epithelium, or from lining alveolar cells remains a mystery or, at least, is controversial.

To this tumor a variety of names has been given. Thus Lohlein¹² called it "cystic papillary lung tumor", Bonne¹ introduced the name of "pulmonary adenomatosis", Taft and Nickerson²⁵ preferred "mucous epithelial hyperplasia of lungs" and finally the term "alveolar cell tumor of lungs" was suggested by Neubuerger and Geever.¹⁵ The term "diffuse primary alveolar carcinoma of lung" is here used for reasons to be discussed.

The diagnosis of this type of tumor has only been made post mortem in all instances, except by Wood and Pierson.²⁷ In their case, the diagnosis was made on surgical material removed at operation. Lobectomy was performed but circumstances subsequently proved that the lesion was diffuse and bilateral.

As far as the author is aware, no clinical diagnosis without recourse to examination of tissue has ever been made. As will be demonstrated, however, it is possible to make the diagnosis ante mortem by biopsy of tissue aspirated from the lung. In the case to be reported, the clinical diagnosis of diffuse alveolar carcinoma of the lung was suspected and discussed, but was not clinically made. In retrospect, the biopsy of aspirated tissue was clearly diagnostic, but a definitive diagnosis was not made, in spite of the author's experience with a previously reported case.²²

REPORT OF CASE

Clinical Data

The patient was a 39 year old man who was first admitted to this hospital, March 5, 1946, in the service of Dr. David Mendel. At this time his complaints were chest cold and

* Received for publication, June 18, 1947.

cough of six weeks' duration, blood-tinged sputum, general malaise, fever and vague pain of twenty-four hours' duration.

Past illness. In November 1931, he was admitted to the Royal Victoria Hospital because of dizziness, loss of consciousness, hematemesis and melena, but no cause for the gastro-intestinal bleeding was discovered at that time. In December 1934, he was again admitted to the Royal Victoria Hospital with a history of melena which had recurred six or seven times since 1931, the last time in August 1934. As on the previous examination, no evidence of disease of the gastro-intestinal tract was found. He was then well until September 1944, when, while working in Vancouver, B. C., he became very weak one morning and passed a black, tarry stool. He was treated in the Emergency Clinic of the Vancouver General Hospital and discharged the same day. He had no further hematemesis or melena. In May 1945, the patient caught a chest cold and complained of severe pain in the left side of the chest accompanied by cough and sputum. X-ray films of his chest revealed a lesion which was suspected of being tuberculous and he was admitted to the Tranquille Sanatorium in Vancouver for nine weeks. Here, repeated examinations of sputum and guinea pig inoculations were negative for tubercle bacilli. An x-ray film taken May 6, 1945 revealed "light infiltration within the second and third interspaces and dense, soft-looking infiltration in the lower hilar area extending to the mid-lung zone on the right and light mixed infiltration in the fourth interspace on the left". He was treated with sulfadiazine and admitted to the Vancouver General Hospital on July 10, 1945. After thirteen days in the hospital, he was discharged with a diagnosis of long-standing pneumonitis.

Present illness. The patient remained in Vancouver until January 1946 when he contracted "flu". The cough continued and he returned to Montreal late in February. He was told by a physician that he had pneumonia and was admitted to this hospital on March 5. On the previous day he had noted that his sputum was tinged red.

Physical examination revealed a moderately well nourished individual lying comfortably in bed. On admission, his temperature was 99.8 F., pulse rate 85 per minute and respiratory rate 20 per minute. The physical findings in the chest were compatible with those of consolidation and pleural effusion on the left side. X-ray films of the chest on March 7, two days after admission, showed large areas of consolidation in the right upper lobe and in the right and left mid-lung fields.

The patient remained in the hospital from March 5 to June 15. During this time, his condition was thoroughly investigated. Repeated x-ray examinations of the lungs showed areas of consolidation in both lung fields which at times seemed to diminish in size. X-ray films of the gastro-intestinal tract and urinary tract failed to reveal evidence of disease. White blood cell counts varied from 7600 to 23,700 per cu. mm. and the sedimentation time was increased. Repeated examinations of sputum for tubercle bacilli and fungi of various types were negative. The patient's temperature fluctuated irregularly between 98 F. and 103.6 F. and he was given large amounts of penicillin with indifferent results. Bronchoscopic examination on March 16 was negative. *Biopsy* of material aspirated from the lungs on May 9 revealed a few atypical cells. The diagnosis stated: "The possibility of tumor cells cannot be excluded."

The clinical diagnosis during this period of the patient's hospitalization was in doubt, the possibilities of fungus infection of the lung and of Boeck's sarcoid having been considered. The patient was discharged on June 15 after 102 days in the hospital with a diagnosis of "chronic lung disease, etiology unknown".

He was re-admitted to the hospital on August 15, 1946. During the preceding two months, he worked occasionally, but continued to have weakness, cough and vague pains in the chest. About July 15, he noticed increased shortness of breath and a week later expectorated blood-tinged sputum and developed chills, fever and sweats. These symptoms became progressively worse until re-admission to the hospital.

Physical examination again revealed irregular signs of consolidation in both sides of the chest. His leukocyte count on admission was 22,500, his pulse rate was 115 per minute, respiratory rate 20 per minute and temperature 102 F. He was critically ill and the tem-

perature swung irregularly from 98 F. to 104.5 F. Repeated x-ray films at this time and throughout this hospital admission revealed evidence of consolidation of both lung fields. These areas appeared to vary in size and shape from time to time, occasionally increasing and sometimes diminishing in size. Clinically, no definite diagnosis could be established.

On August 21 a lung puncture was performed and the following report was made from examination of a *microscopic section* of the aspirated material (Fig. 2): "Atypical epithelial cells, probably tumor cells. Papillary masses of glandular epithelial cells in the tissue section strongly suggest a tumor of adenomatous type."

Another lung puncture on September 4, failed to reveal the presence of tumor cells. After twenty-six days of fever, the patient's temperature became normal. Numerous examinations of sputum and aspirated lung material for fungi were negative. Type III *Pneumococcus* was recovered from a lung puncture on one occasion. His white blood cell count dropped to 8900 per cu. mm. and penicillin was discontinued. The patient was discharged, slightly improved on October 29, seventy-four days after admission with a clinical diagnosis of chronic and organizing interstitial pneumonitis. It was during this admission that the possibility of a diagnosis of diffuse alveolar carcinoma was considered. During this hospitalization Dr. N. Freedman also reported the presence of peculiar cells in the sputum. These were large, oval cells and were unlike any he had ever seen before in sputum. The cells were shown to Dr. George Papanicolaou who could not identify them but considered them to be tumor cells.

Nine weeks later, on January 3, 1947, the patient was re-admitted to this hospital for the third and last time. During the preceding four weeks his cough had increased in frequency and blood-streaking of sputum had become more frequent. He developed attacks of unconsciousness with deviation of the eyes and intermittent attacks of severe headaches. Six days before admission weakness of the left foot and leg were noted. Physical examination at this time again revealed signs of irregular consolidation in both lungs and a hemiparesis involving the left side of the body.

X-ray findings were essentially the same as previously reported. On neurologic consultation, it was suggested that there was a lesion of indeterminate type in the right cerebral cortex. The patient had a number of epileptiform seizures while in the hospital. His condition deteriorated rapidly and a sputum examination on January 7, revealed cells which could not be excluded as tumor cells. He died on January 26, twenty-three days after his last admission. Although numerous conditions were considered, including pulmonary adenomatosis, no definite clinical diagnosis was established.

Macroscopic Postmortem Findings

Autopsy was performed sixteen hours after death.

The right pleural cavity was completely obliterated by dense, sheetlike, firm adhesions which bound the lungs on all aspects to adjacent structures including the pericardial sac and diaphragm. The right lung was voluminous and within it irregular and indefinite masses could be palpated. The lung had a rubbery consistency and at the apex a few emphysematous areas were present.

A few dense, sheetlike adhesions bound the left lung to the lateral chest wall. Dense adhesions also bound this lung to the diaphragm and pericardial sac. Where adhesions were not present, the pleural surfaces were smooth and of a mottled violet and gray color. Within the lung, which was emphysematous, irregular and indefinite rubbery masses could be palpated in both lobes. The right lung weighed 1670 gm. and the left 1450 gm.

Multiple sections through the lobes of the right lung revealed cut surfaces that were largely replaced by pinkish gray, finely granular, gelatinous and translucent tumor masses (Fig. 1). These tumor masses varied from 0.3 to 3.5 cm. in diameter. The edges were poorly defined and the tumor appeared to merge insensibly into the adjacent lung parenchyma. These nodules were raised slightly above the general level of the lung. Some were discrete, others had coalesced and, when scraped, showed a glairy, gelatinous and sticky material on the knife. In some places in the intervening lung tissue between the tumor masses



FIG. 1. Right lung showing nodular, diffuse character of tumor. The cut surface presents a sticky, gelatinous appearance. Print from a colored lantern slide.

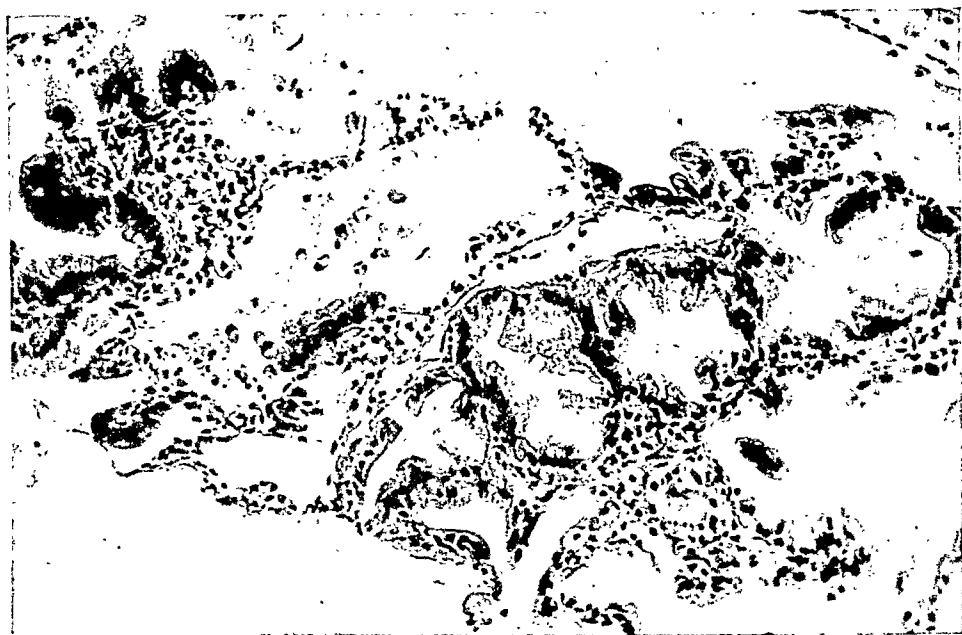


FIG. 2. Material aspirated from lung during patient's second hospitalization. This is a fragment of lung with alveoli lined by tall, columnar, epithelial tumor cells showing papillary infolding. Hematoxylin and eosin. $\times 185$.

dilated alveoli could easily be seen with the naked eye. These changes were present in all lobes of both lungs with variation only in the size and configuration of the tumor nodules and in the degree of emphysema and hyperemia of the intervening lung.

Sections through the trachea and major bronchi revealed a pink, velvety and intact mucous membrane. The terminal portions of the bronchi in the lower lobes were slightly dilated and contained reddish brown, mucoid material. Careful search in the multiple bronchi examined failed to reveal any ulcerating or stenosing lesion which might suggest a primary bronchiogenic tumor.

The paratracheal, mediastinal and peribronchial lymph nodes were distinctly enlarged and measured up to 2.5 cm. in diameter. On section, they presented moist, mottled gray and black, homogeneous surfaces with no gross evidence of tumor.

Painstaking search of all other thoracic and abdominal viscera failed to reveal gross evidence of any tumor. No lesions were found in the gastro-intestinal tract to explain the patient's previous melena (1931 to 1944).

The right cerebral hemisphere of the brain was larger than the left. In the precentral region of the vertex of the right cerebral hemisphere and extending along the median fissure was a sharply demarcated area of diminished consistency which measured approximately 5.0 cm. in greatest diameter. A similar less sharply defined area was present in the paracentral region at the vertex on the left side. Coronal sections of brain (Fig. 4) revealed these two areas to be more or less cystic or honey-combed in appearance and some of the cysts were filled with colorless, gelatinous material. A third metastasis was present in the right cerebral hemisphere, more caudad and at the level of the pons.

Microscopic Findings

Many blocks were taken from every lobe of the lungs to include obvious tumor, peripheral portions of tumor and grossly uninvolved pulmonary tissue. The histologic changes were essentially similar in all areas except in the firmer masses where considerable interstitial fibrosis of the lung was noted microscopically.

Throughout all sections, large areas were encountered where thin-walled, slightly dilated alveoli were lined by single and, at times, pseudostratified layers of tall, columnar epithelial cells. These cells were loosely attached to apparently unaltered alveolar walls and in many places were detached in small strips or sheets and lay free within alveoli or were attached to the alveolar wall only at one end of the strip. Papillary infolding into alveoli was commonly observed.

The lining cells showed basally arranged, uniform, oval nuclei composed of finely granular chromatin. Moderate numbers of nuclei showed rather prominent nucleoli but this was not a constant feature (Fig. 5). Only rarely was a mitotic figure seen. The tumor cells showed abundant pale pink-staining cytoplasm and many of the cells contained "goblets". From these "goblets" small amounts of pale mucoid material extruded and was found free within alveoli. This mucoid material stained pink with hematoxylin and eosin. In our hands Best's mucicarmine stain gave indeterminate results and with the Masson trichrome stain the mucoid material stained a pale blue. The tumor cells showed no cilia but a fine "brush border" was noted in the better preserved cells.

Many alveoli were found filled with desquamated tumor cells and, in some alveoli, large, pale, circular cells were seen showing finely vacuolated cytoplasm and relatively small compact nuclei. Transitional forms between obvious desquamated tumor cells and these phagocytic cells were noted. Fields were encountered in which only rare alveoli were lined by a few desquamated tumor cells and here the majority of the alveoli contained fairly large numbers of lym-



FIG. 3. Low power view of lung. Note large area of organized interstitial pneumonia, lower left, containing residual alveoli lined by tumor cells. The diffuse nature of the tumor involving the alveoli is seen about the area of fibrosis. Hematoxylin and eosin. $\times 25$.

phocytes and occasional polymorphonuclear leukocytes. No fibrin or bacteria were noted. Cultures from these lungs at autopsy were sterile. /

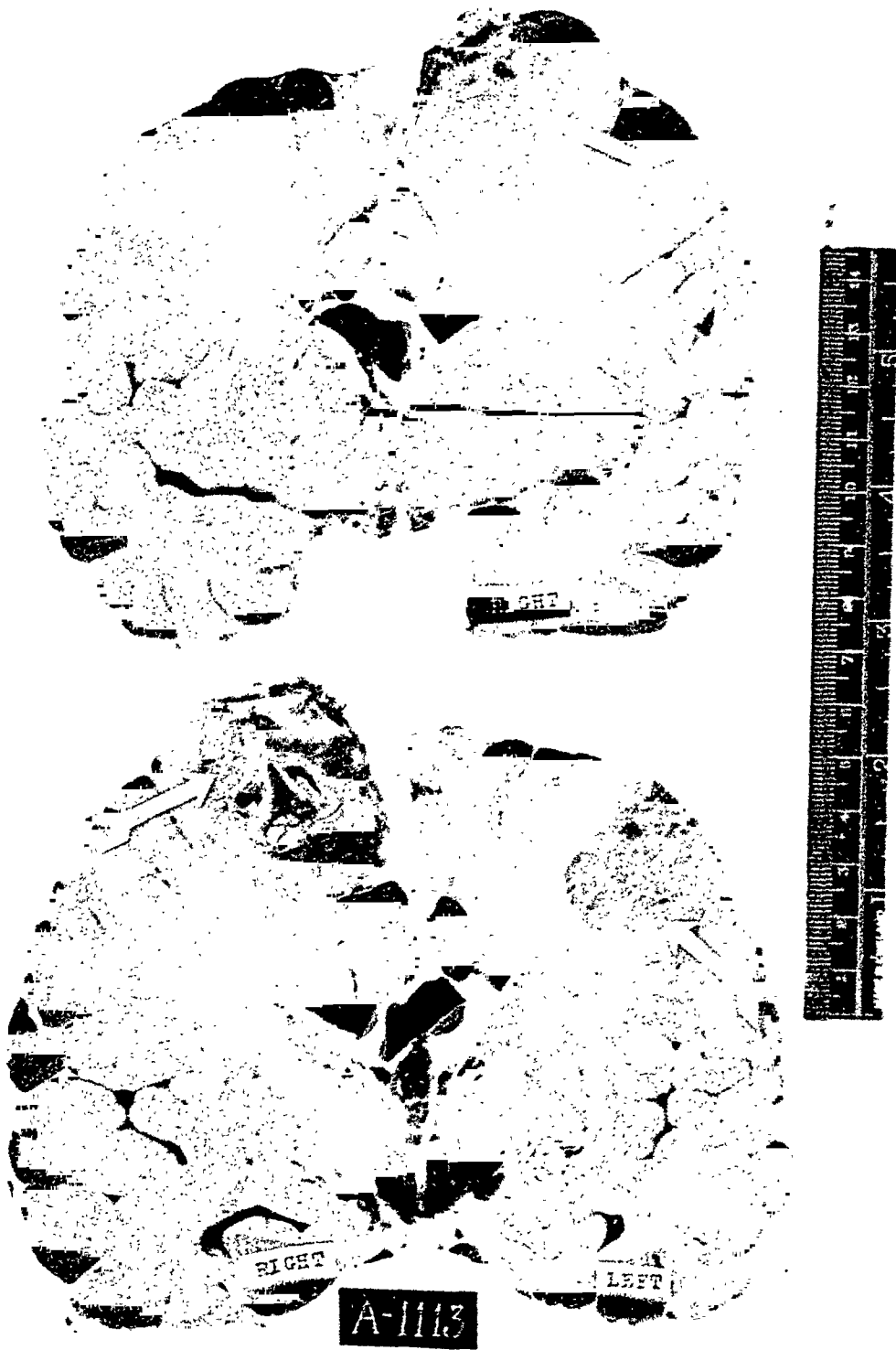


FIG. 4. Coronal sections of brain showing metastases. Note the large cystic areas filled with clear, mucoid material.

In all lobes except the right lower lobe, extensive irregular areas of fibrosis were encountered. These areas (Fig. 3) were composed of compact and relatively cellular connective tissue within which were encountered a few irregular spaces lined by cells somewhat shorter than those lining the unaltered alveoli.

The lining cells in these areas were regular in appearance and basement membranes were sharply defined. The general impression gained from such areas was that these spaces represented residual alveoli in foci of organized interstitial pneumonitis. In the periphery of these areas of fibrosis the alveolar walls appeared to be unaltered and here alveoli were lined and partly filled by typical tall columnar epithelial tumor cells.

Throughout all sections painstaking search failed to reveal any identifiable bronchioles which were lined by similar tall tumor cells. Wherever bronchioles were identified stratified cuboidal cells were present differing in no respects from those usually found. In all sections nothing was seen that could be identified as lymphatic or blood-vascular invasion.

Sections through multiple hilar and mediastinal lymph nodes failed to reveal microscopic evidence of metastatic tumor.

Sections taken from the region of the cystic areas in the cerebral hemispheres (Fig. 6) showed extensive replacement and destruction of brain tissue by tumor identical to that described in the lungs. Here large cystic dilated spaces were seen lined by simple and pseudostratified, nonciliated columnar epithelium showing papillary infolding. Into some of these cystic spaces desquamation of lining cells had occurred and, within these spaces, spherical cells with vacuolated cytoplasm and eccentric nuclei similar to those found within alveoli were noted. Many of the spaces contained pink-staining, stringy mucoid material identical with that seen in the alveoli lined by tumor cells. Adjacent to the tumor which invaded the cortex and subjacent white matter, there was extensive destruction of brain tissue with compound fat granule cells and microglial cells present in large numbers. The tumor extended into the leptomeninges where the cysts appeared to be larger than elsewhere.

Sections taken through all other organs failed to show microscopic evidence of tumor.

Anatomic Diagnoses

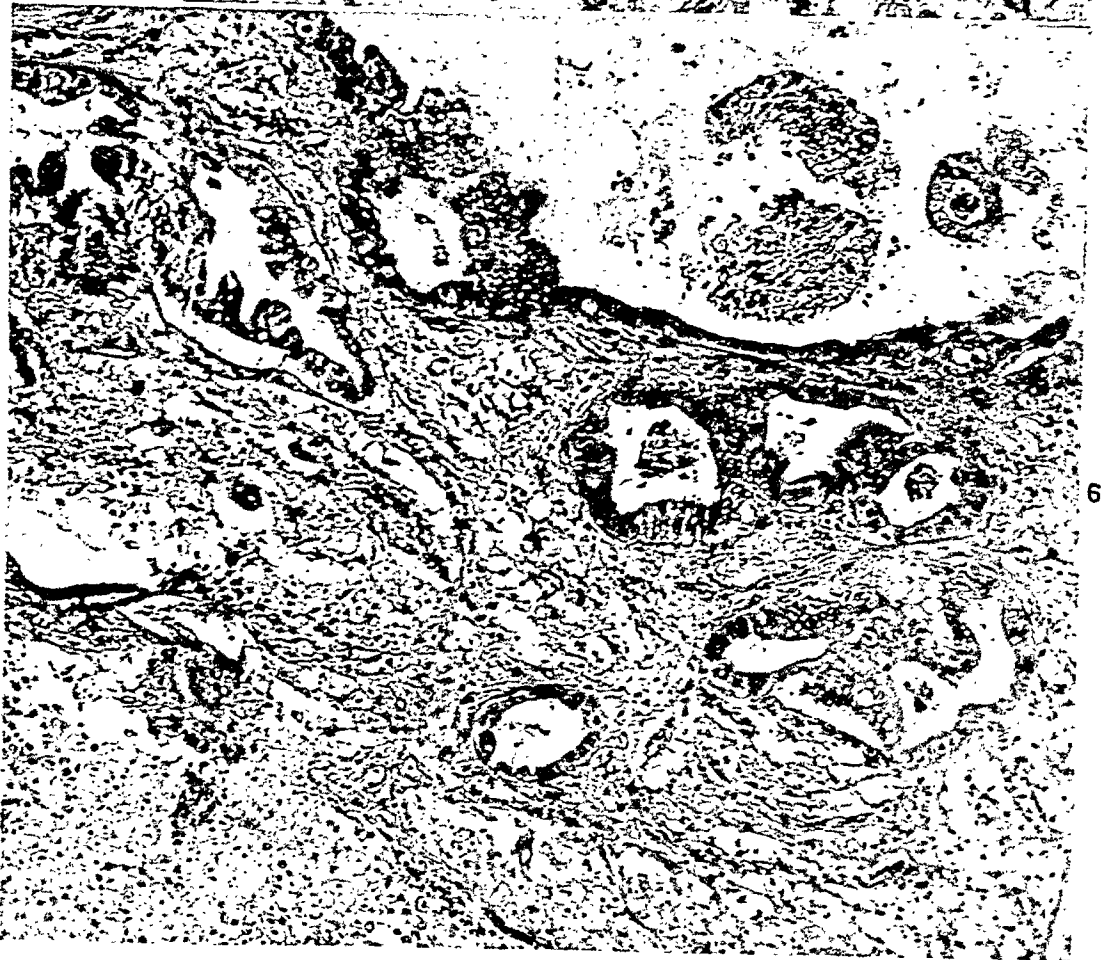
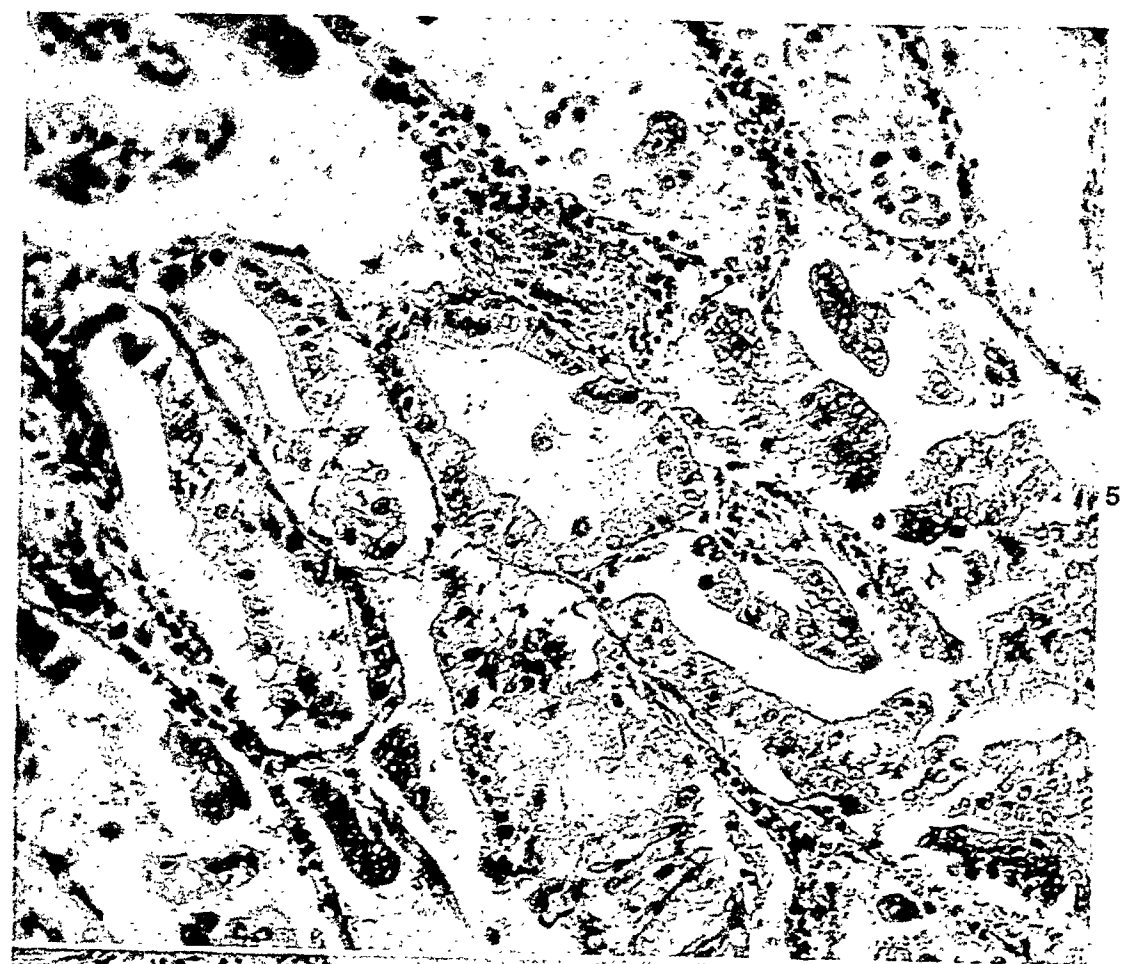
Diffuse primary alveolar carcinoma of the lungs, bilateral, with metastases to brain; organizing interstitial pneumonitis, bilateral; bronchopneumonia, bilateral.

DISCUSSION

Diffuse primary alveolar carcinoma of the lungs may present two distinctly different gross pictures. In one form, the gross picture may be indistinguishable from lobar pneumonia in the state of so-called gray hepatization. Here the

FIG. 5. Medium-powered section of lung to show character of lining cells, unaltered alveolar walls and brush border of cells. Note detachment of cells in alveolus to left of center and desquamated cells in alveolus in upper and lower left corners. Goblet formation can also be seen in many cells. Hematoxylin and eosin. $\times 200$.

FIG. 6. Metastasis in brain. Note that the cells lining the acini are identical with those seen in the lung with the same tendencies to papillary formation, desquamation and mucoid production. Note compound fat granules, lower left. Hematoxylin and eosin. $\times 93$.



lung is gray and consolidated but may present a sticky, mucoid substance on cut surface. In a previously reported case²² which was of "pneumonic" type, Friedländer's bacillus was recovered. Taft and Nickerson²⁶ have likened the cut surfaces of the lungs of diffuse primary alveolar carcinoma to Friedländer's pneumonia. In the present case, Type III *Pneumococcus* was recovered during life.

In the second form, the lungs of diffuse primary alveolar carcinoma may present a nodular appearance similar to that in the present case (Fig. 1). In this form, the cut surface shows numerous large and small, poorly defined, elevated, pinkish gray masses which blend imperceptibly with the surrounding lung tissue. The surfaces of these nodules are, at times, finely granular and are covered with a sticky, mucoid material and the intervening lung tissue shows variable degrees of hyperemia. These nodules may be rubbery in consistency and the intervening lung shows variable degrees of crepitation depending on the amount of emphysema and/or pneumonia present. In this connection it may be pointed out that a pneumonia of variable degree always accompanies diffuse primary alveolar carcinoma of the lungs and may be of lobar type as in the case of Alexander and Chu.¹ In some cases variable amounts of organized interstitial pneumonitis may be the residua of former pneumonic processes.

Microscopically, the picture is characteristic. Alveoli, the walls of which are apparently unaltered, are lined by delicately attached, single, or pseudo-stratified layers of tall, columnar epithelial cells many of which show papillary infolding into the alveoli. These cells are regular in appearance, size and staining quality with basally arranged oval nuclei. The nuclei show finely granular chromatin, occasional nucleoli and, only rarely, is a mitotic figure encountered.

The lining cells frequently show "goblet" formation and produce a type of secretion which we have found does not stain readily with Best's mucicarmine stain. Alexander and Chu¹ stated they were able to stain the mucinous material by this method and Taft and Nickerson²⁶ mentioned mucus which stained with "aniline dyes". In any event, an evident mucoid material appears to be secreted by these cells and may also be found in the alveoli. The tumor cells are not ciliated but may show a fine "brush border" at the free edge. The lining cells desquamate as single cells or sheets of cells into the alveoli where they may assume circular forms. At times, it appears that the large, pale phagocytic cells seen in many of the alveoli are altered desquamated tumor cells. These lining cells in no situation appear to be continuous with recognizable lining bronchiolar cells either in random or serial sections.

The distribution of these lining cells is quite irregular. Some microscopic fields show all alveoli to be lined by tumor cells and other fields reveal no alveoli thus lined. All lobes of the lungs are involved in variable degree. In some instances, where interstitial fibrosis is found in the lungs as the result of organizing pneumonitis, numerous spaces, probably residual alveoli, are found lined by tumor cells (Fig. 3).

Lymphatic invasion is rarely observed and blood vascular invasion is even more rare. These must occur, however, in order to explain the metastases.

In the larger group of so-called "alveolar cell tumors of the lungs" collected by Neubuerger and Geever¹⁸ the lining cells have been shown to vary from high cuboidal to columnar and much anisocytosis and considerable degrees of anaplasia have been noted. In their collected cases no communication or continuity between lining cells and bronchiolar epithelium has been established.

Histogenesis

First and foremost, in this case as in almost all other similar cases reported in the literature, the ordinary criteria suggesting a primary bronchial origin were absent. (Ikeda's¹² case No. 2 showed a gross bronchial tumor and therefore is probably unacceptable as an example of the type of tumor under discussion.) There was no gross or microscopic invasion of bronchial wall either with or without stenosis or ulceration and no associated atelectasis was present. Indeed, the character and appearance of the cells lining the alveoli in cases of diffuse primary alveolar carcinoma are so unlike the ordinary bronchiogenic carcinomas as to suggest at once that the tumors are different. The lining of unaltered alveolar walls by these loosely attached cells in ordinary bronchiogenic carcinoma (if it exists) certainly must be most unusual.

Are these tumors in the lung metastatic in origin? To this question a categorical negative answer can be given. In almost all the cases reported in the literature, as in the present case, no primary tumor was found other than in the lungs. Since there are now about 70 recorded cases it seems extremely unlikely that more than 60 pathologists would all miss a primary tumor in a situation other than the lung. It must be admitted, however, that occasionally a metastatic carcinoma may assume an intra-alveolar arrangement not unlike that presented here. This is an exception rather than the rule and does not appear to deserve the emphasis placed upon it by Herbut.¹¹

Herbut¹¹ has also suggested that these tumors arise from bronchiolar epithelium particularly in foci of bronchiectasis. This concept cannot be substantiated by actual gross or microscopic examinations. In the first place, bronchiectasis in diffuse alveolar carcinoma of the lungs is an extremely infrequent and inconstant accompanying condition. Numerous cases of diffuse alveolar carcinoma have been reported in which the lesion has been bilateral and has showed no evidence of lymphatic invasion or lymph node metastases. Under these circumstances, if Herbut¹¹ is correct, bilateral and diffuse bronchiectasis should be of frequent occurrence in this condition. The supposition that the tumor may start in one focus and then by continuity line pre-formed spaces such as the alveoli in widely separated portions of the lung without in some places lining bronchioles or bronchi seems untenable unless one assumes implantation by aspiration. In microscopic sections it has been impossible to demonstrate direct continuity either in random or serial sections between bronchiolar epithelium and tumor cells lining the alveoli. Furthermore, the cells lining bronchioles are quite different from the tumor cells, none of which ever shows cilia. For these reasons it appears unlikely, no matter how tempting the concept, that the tumor cells arise from bronchiolar lining cells.

The inability to demonstrate that these tumors arise from bronchiolar lining cells immediately raises the controversial question as to whether epithelial alveolar lining cells exist, and if so, whether tumors may arise from these cells. Such histologists as Bensley and Bensley,³ Miller,¹⁶ and Cooper⁵ are of the opinion that a continuous layer of epithelial cells lines the alveoli. Maximow and Bloom,¹⁵ Rose,²⁰ Fried,⁸ and Loosli¹⁴ believe that the lining cells are mesenchymal. Ross²¹ believes that both mesenchymal and epithelial cells may line alveoli. It is difficult to imagine that the lung which is a diverticulum of the primitive gut should differ from other evaginations, all of which are lined throughout with epithelium.

Bell² and Geever, Neubuerger and Davis⁹ studied the lung as affected by chronic passive hyperemia and by inflammatory conditions. They observed what they considered to be epithelial proliferation from alveolar lining cells. Hence, it was their opinion that alveolar lining cells may give rise to tumors. Ikeda¹² and Simonds and Curtis²³ have produced alveolar epithelialization in experimental animals by the use of oily substances and of coal tar. Murphy and Sturm¹⁷ and, more recently, Grady and Stewart¹⁰ have induced multiple adenomatous tumors originating along alveolar walls of the lungs of mice, and Slye, Holmes and Wells²⁵ have reported spontaneous, primary, adenomatous tumors of this type in the lungs of mice. The disease known as jagziekte, epizootic adenomatosis, or verminous pneumonia, an infectious endemic disease of sheep, the virus of which was recently isolated by Dungal,⁷ is regarded as an adenomatous hyperplasia of the alveolar epithelium by Cowdry and Marsh.⁶

The experiments and observations quoted immediately above strongly suggest that alveolar epithelium does exist and that under proper circumstances may proliferate and may, indeed, give rise to tumors. The fact that cases have been described in which the tumor was localized to the lungs without gross or microscopic evidence of lymphatic invasion tends to support the concept of a multicentric origin of this type of tumor.

Because of the controversy concerning the existence of epithelial alveolar lining cells, considerable confusion in the nomenclature of this tumor has arisen in the literature. Neubuerger and Geever¹⁸ chose the name "alveolar cell tumor" without intending to connote the histologic origin of the cell. If the word "cell" is omitted and the condition described as "diffuse primary alveolar carcinoma of the lungs", definitive reference as to possible cell origin (*e.g.*, bronchial, infundibular or alveolar) is omitted in this perplexing subject. Similarly, the terms "pulmonary adenomatosis" and "mucous epithelial hyperplasias of the lung" are misleading for, in spite of the benign appearance of some of these tumors histologically and, particularly when they are, as yet, confined to the lungs, this tumor should be regarded as a slow-growing but eventually metastasizing carcinoma. Invariably there is a considerable degree of pneumonia associated with these tumors and it may well be that the degree of pneumonia frequently is the cause of death before metastases have had time to develop.

The etiology of diffuse primary alveolar carcinoma of the lungs in man is unknown. From sheep affected with jagziekte, Dungal⁷ isolated a virus which produces a strikingly similar lesion in the lungs of these animals. This virus does

not appear to be pathogenic for man as shepherds who are housed for long periods with these sick animals do not contract the disease. Attempts to transmit the disease to various laboratory animals with human autopsy material by Richardson,¹⁹ Sims,²⁴ and Wood and Pierson²⁷ have been uniformly unsuccessful.

The clinical course and clinical findings in diffuse primary alveolar carcinoma of the lungs are not sufficiently clear-cut to permit an unequivocal clinical diagnosis. Cough, x-ray evidence of bilateral lung consolidation which varies in size from time to time, absence of eosinophilia (Loeffler's syndrome should be excluded), with or without hemoptysis and the progressive nature of the disease, are suggestive features. The diagnosis, however, can be established during the life of the patient on the basis of biopsy of material aspirated from the lung. In this case the diagnosis should have been established on the basis of such a biopsy (Fig. 2).

SUMMARY

A case has been presented of diffuse primary alveolar carcinoma of the lungs with metastases to the brain. This disease has also been called "pulmonary adenomatosis", "mucous epithelial hyperplasia of the lungs" and "alveolar cell tumor of the lungs". Although controversial, the evidence suggests that this tumor is multicentric in origin and is probably derived from lining alveolar epithelial cells. Clinically, the signs and symptoms of this disease are not sufficiently characteristic to permit a correct diagnosis but a correct diagnosis can be established during the life of the patient on the basis of biopsy of material aspirated from the lung.

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ENDEMIC (MURINE) TYPHUS.

REPORT OF AUTOPSY FINDINGS IN THREE CASES*

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Endemic (murine) typhus, which is now seen frequently in southeastern United States, was separated epidemiologically from Old World epidemic typhus by Maxcy⁷ in 1926. He suggested a possible rat flea vector. Dyer,³ in 1931, showed experimentally that rat fleas could transmit the disease. Although this disease differs clinically, epidemiologically and etiologically (*Rickettsia prowazeki mooseri*)² from other rickettsial diseases, and although the reported mortality rate in the United States varies from 1 to 4 per cent, no detailed autopsy reports of persons in the United States dying from this disease have been published. Morbidity reports for endemic typhus in the United States for 1944 included 5353 cases.

In 1931, Pinkerton and Maxcy⁹ made a pathologic study of a case diagnosed as typhus near Charlottesville, Virginia. Later, after the eastern type of Rocky Mountain spotted fever was recognized, it was decided that death was due to that disease rather than to typhus (personal communication from Dr. Pinkerton).

In a discussion of 56 cases of endemic typhus from Texas, Kemp⁵ briefly mentioned one autopsy and stated that no definite myocardial damage was demonstrable and that other autopsy findings were not significantly different from previous descriptions.

In view of the absence of detailed autopsy studies on cases of endemic typhus occurring within the United States, it seems worthwhile to report the observations made on material from three autopsies. Each case will be reported separately; no attempt will be made to describe the general pathology of the disease in such a small series, nor will there be any comprehensive attempt to compare the pathologic changes in these few cases with those classically described from a large number of cases of epidemic typhus,¹¹ Rocky Mountain spotted fever⁶ and scrub typhus.^{1, 8}

REPORT OF CASES

Case 1

Clinical data. V. J. B., a white man, aged 46, a motion picture machine operator in a New Orleans theater, was admitted to the Medical Service, U. S. Marine Hospital, New Orleans, Louisiana, on March 30, 1944. Illness had begun four days previously with chills and fever. These symptoms were followed by headache, nausea, vomiting and pain in the left upper quadrant of the abdomen. His family physician had treated him with atabrine and quinine.

* Received for publication, June 30, 1947.

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The significant observations made on admission were an enormously enlarged, tender spleen, epigastric tenderness and a macular skin eruption over the trunk and upper extremities.

The essential laboratory data included negative Kolmer and Wassermann tests, mild albuminuria, a negative blood culture, negative findings in the cerebrospinal fluid, 9.6 gm. hemoglobin, 2,800,000 erythrocytes, 67,000 platelets and a normal clot retraction. The leukocyte count, which was 3400 on admission, decreased progressively and was 1200 on the eleventh day of disease. The neutrophils ranged from 58 to 44 per cent. Sternal marrow findings were interpreted as showing maturation arrest of the granulocytes. The icterus index was 225 on the ninth day and 90 on the twelfth day of illness. Numerous smears were negative for malarial parasites. Agglutination for typhoid and paratyphoid antigens and *B. abortus* were negative. Agglutination titers for Proteus OX19 (Welch and Stuart antigen) rose rapidly from 0 on the fifth day of disease to positive in dilution 1:320 on the tenth, 1:640 on the eleventh, 1:1240 on the thirteenth and 1:1940 on the fifteenth day of the disease. Complement-fixation tests with rickettsial antigen* gave positive results with endemic typhus antigen in dilutions of 1:64 on the fifth day and 1:512 on the tenth day. Results with epidemic and Rocky Mountain spotted fever antigens were negative.

The temperature curve was of the septic type, ranging from 99 F. to 104 F. The skin rash persisted and spread to include the forearms, palms and soles. Hemorrhages occurred in the mucous membranes of the oral cavity. Icterus and meningismus were noted on the eighth day of disease. Although no malarial parasites were demonstrated, atabrine and quinine were again started at that time without beneficial effect. Death occurred on the fifteenth day of illness.

About the eighth day of illness two guinea pigs were inoculated intraperitoneally with whole blood to rule out Weil's disease. Fortunately, one animal was male, and on the ninth day exhibited severe reddening and swelling of the scrotum.

Postmortem findings. The examination was begun four hours after death. The skin showed jaundice and poorly defined mottling over the shoulders, upper arms and upper thighs. The right lung weighed 520 gm. and the left, 540 gm. The left lower lobe exhibited a patch of fibrinous exudate on the pleural surface about 2 cm. in diameter, and beneath this the parenchyma for a depth of 1 cm. was firm and yellow. This lobe contained a more deeply placed, firm, reddish nodule 2 cm. in diameter. The heart weighed 370 gm. The right ventricle was moderately dilated, but otherwise no abnormalities were observed. The liver was enlarged to 2770 gm. The capsule was smooth, the color brownish pink and the lobules were not well defined. The gallbladder contained little bile and the biliary ducts were all patent.

An enormously enlarged spleen weighed 1100 gm. The capsule was smooth and the pulp, reddish. Two regions, about 2 cm. and 5 cm. in diameter, respectively, presented a homogeneous appearance suggesting infarction. The gastro-intestinal tract appeared normal. The lymph nodes about the pancreas and abdominal aorta were enlarged to a maximum size of 3 x 2 x 2 cm. They were pale pinkish gray in color and very soft in consistency. The right kidney weighed 300 gm. and the left, 270 gm. The cortex of each kidney was swollen. The brain weighed 1700 gm., but revealed no changes, except for swelling and congestion. The other viscera showed no changes worthy of note.

Microscopic findings. The epicardium and myocardium were diffusely infiltrated by large mononuclear cells, plasma cells and lymphocytes. There was some tendency toward perivascular distribution about the larger vessels but the infiltrate was chiefly seen between the muscle fibers. The endothelium of the epicardial capillaries was slightly swollen, but vascular obliteration was not observed.

* Complement-fixation tests were performed by Dr. Ida Bengston, National Institute of Health, Bethesda, Maryland.

In general, the *lung* sections revealed mild perivascular mononuclear cell infiltration and slight widening of septa by such infiltrates. One of the subpleural nodules seen at autopsy revealed necrotizing fibrino-cellular exudate and degenerating septa. Bordering this necrotic area, alveoli contained exudate with fibrin and mononuclear cells and septa were widened by infiltrating mononuclear cells.

A retroperitoneal *lymph node* exhibited considerable disruption of architecture. Conspicuously widened sinuses were engorged with macrophages which often showed erythrophagia and recognition of follicles was frequently difficult. Interstices present in pulp stroma probably represented edema spaces. Perinodular mononuclear cell infiltration was prominent.

In the *liver* there was widening of the periportal stroma by edema, lymphocytes, large mononuclear cells and dilated capillaries. Greatly widened sinusoids contained erythrocytes, a medium number of large mononuclear cells, a few neutrophils and conspicuous Kupffer's cells. Liver cells were compressed and showed fine vacuolization, some bile pigment, but no necrosis. No bile was retained in the ducts or canaliculi.

The follicles of the *spleen* were largely disorganized. There was diffuse sinus endothelial proliferation, engorgement of some sinuses by blood, and in the pulp, numerous mononuclear cells of various sizes. Here also, neutrophils were rare and erythrophagia was rarely seen. A minute (1 mm.) focus of necrosis was seen in one section.

The tubular epithelium of the *testis* was considerably disrupted and edematous. The lumens often contained a cellular debris of poorly defined cells with pyknotic nuclei and wide, granular, cytoplasmic zones. In the interstitial tissues there was a patchy infiltrate of lymphocytes, plasma cells and large mononuclear cells. The epithelium of the epididymis was not unusual but the interstitial infiltrate resembled that seen in the testis.

The *scrotum* exhibited a focal, necrotizing lesion with ulceration. The corium was edematous. Capillaries occasionally were obliterated by swollen endothelial cells and fibrin and sheathed by clusters of mononuclear cells, some with wide amphophil cytoplasmic zones. An occasional endothelial cell or pale mononuclear cell contained minute basophil (Romanowsky stain) diplococcoid bodies.

In sections of vertebral *bone marrow* stained by Romanowsky technic very few granulocytes could be identified. Between the sinuses a meshwork of oval or spindle cells formed a supporting stroma for rounded, oval or distorted cells with generally leptochromatic nuclei. Among these cells were many pyknotic nuclei. The erythrocyte series was focally hyperplastic. Erythrophagia was prominent. Megakaryocytes were conspicuously increased in number but exhibited various degrees of necrosis and many shadow forms.

The microscopic changes in the *brain* were not severe. Only rarely did a vessel of the meninges or outer cortex exhibit endothelial swelling and mild perivascular mononuclear cell infiltrate. A very mild mononuclear cell infiltration of the meninges was seen. Generally there was hyperemia, but no other

changes of note were observed. The pituitary capsule was infiltrated by mononuclear cells, but no parenchymal changes were seen.

Striated muscle (rectus) showed no necrosis. There was mild pericapillary and interstitial mononuclear cell infiltration. Capillary endothelial cell swelling with occasional capillary obliteration was seen.

The *kidneys* exhibited dilated capsules and tubules, an occasional bile-stained cast and rare interstitial infiltrate of mononuclear cells.

Aorta, gallbladder, stomach, colon, small intestine, adrenals, urinary bladder, prostate and thyroid gland showed no significant lesions.

Case 2

Clinical data. C. W. O., a white man, 54 years old, a veteran of World War I, was admitted to the U. S. Marine Hospital, Mobile, Alabama, September 8, 1944, on the service of Dr. J. A. Oshlag. He had been working as a special detective in an old, rat-infested building. He had been treated for asthma for many years and had also received treatment for syphilis of the central nervous system, but the findings of the cerebrospinal fluid had been reported normal in 1943.

The present illness began eight days before admission. While in bed at night, he was suddenly seized by a severe pain over the left kidney area which radiated toward the left groin. He worked thirteen hours the next day but returned home exhausted. On the following day he fell asleep in a chair while at work. On the fourth day the family physician prescribed sulfathiazole which, after a twelve hour trial without effect, was discontinued by his wife. On the sixth day, fever, mental confusion and a skin rash were present.

Physical examination revealed an obese, dyspneic man with a generalized macular rash over the legs, trunk, arms and posterior portions of the neck. Respiration was rapid and expiratory wheezing was prominent. The heart borders were not well defined by percussion, and tones were not satisfactorily heard. The blood pressure was 130/76, respiratory rate 26 per minute and pulse rate 120 per minute. Tenderness was elicited over the liver area and the edge of the liver was questionably palpated about 3 cm. below the costal margin. Pitting edema of the ankles was noted. The clinical impression on admission was typhus fever.

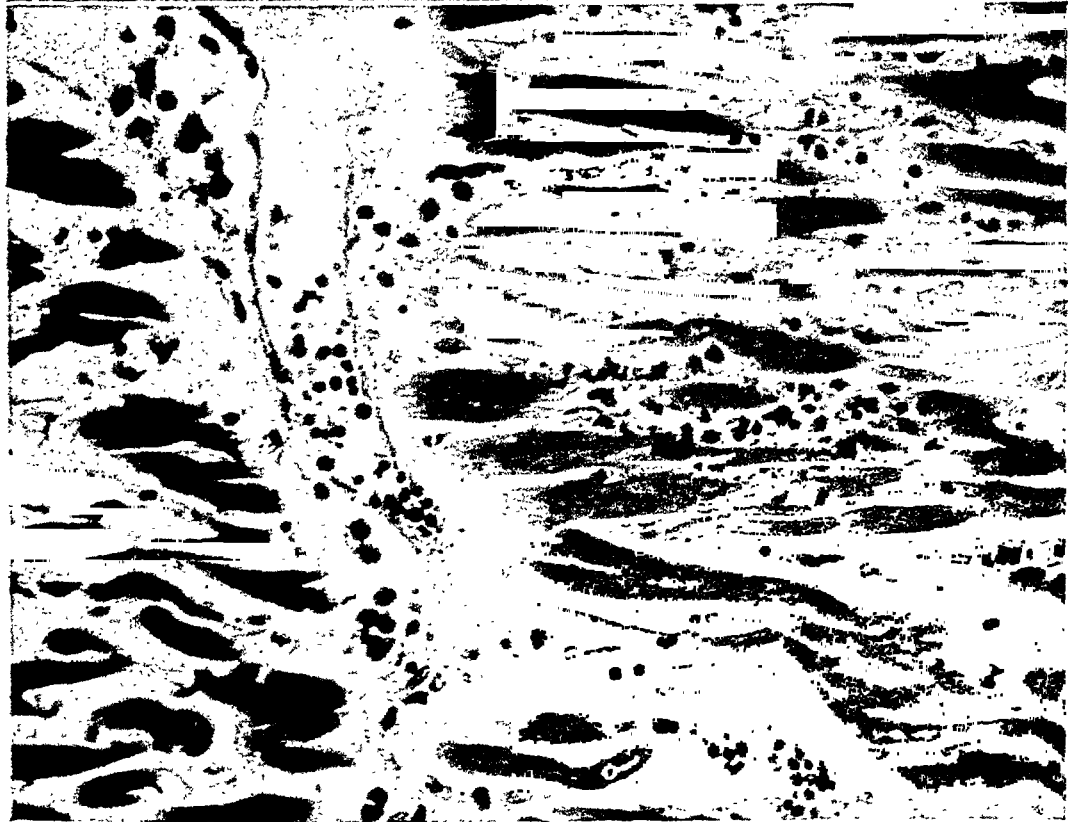
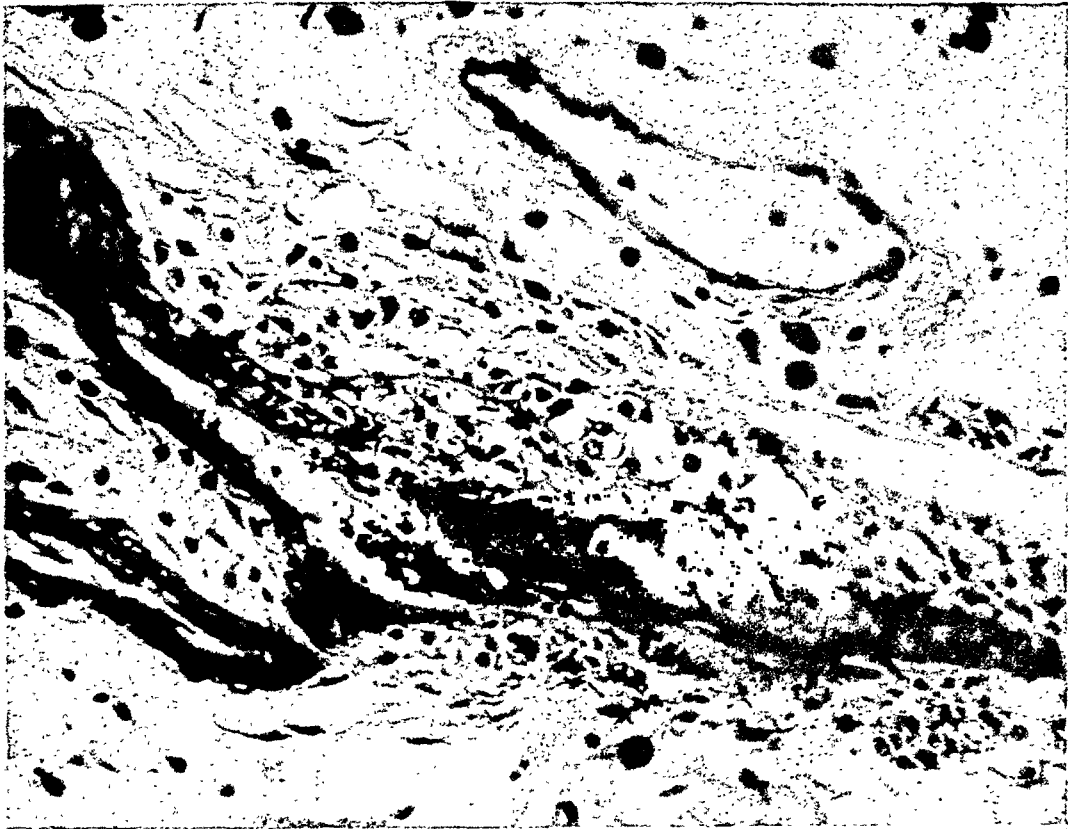
The day after admission the erythrocytes numbered 3,830,000, the leukocytes, 5800, and the hemoglobin level was 80 per cent. A differential count showed 84 per cent neutrophils, 14 per cent lymphocytes and 2 per cent monocytes. Urinalysis revealed a specific gravity of 1.012, no sugar and a trace of albumin. Study for malarial parasites was negative. The nonprotein nitrogen of the blood was 48 mg. per cent. Agglutination studies carried out for members of the typhoid-paratyphoid group and for *B. abortus* were negative. On admission, the Proteus OX19 agglutination (Welch and Stuart antigen) was negative (eighth day of illness), and positive in dilution 1:40 on the third hospital day (eleventh day of illness). The blood serologic test for syphilis was not reported owing to a laboratory accident.

On the second hospital day, sulfadiazine therapy was begun; the total dosage amounted to 8 gm. Sedatives, aminophylline, nicotinic acid and Vitamin B₁ were given liberally. Atropine gr. 1/100 was given every four hours. A roentgenogram on the third hospital day revealed moderate congestion of the bases of the lungs. The temperature ranged from 101.5 F. to 105 F., the pulse rate from 120 to 140 per minute and the respiratory rate, from 40 to 48 per minute.

The patient remained unconscious until his death on September 13, 1944, the fifth hospital

FIG. 1. Case 3. Blood vessel of dermis showing thrombosis and perivascular mononuclear cell infiltrate. $\times 350$.

FIG. 2. Case 3. Myocardium, left ventricle. $\times 350$.



day, and approximately thirteen days after his first symptoms. The final clinical diagnosis by the hospital staff was endemic (murine) typhus. That opinion was shared by the local health officer. Proteus OX19 agglutination on postmortem plasma was positive in dilution 1:160.

Postmortem findings. Autopsy was performed by Dr. Giarver. Examination was begun six hours after death. Only the significant observations are included here. There was basal pulmonary edema, the right lung weighing 950 gm. and the left 970 gm. The apex of each lung was the site of fibrous adhesive pleuritis. No consolidation was recognized. The heart weighed 320 gm., and except for thickening of the leaflets of the mitral valve, no lesions were observed. The liver weighed 1900 gm. and the spleen, 200 gm., but no unusual features were noted. The right kidney was the site of simple cortical cysts. The other abdominal viscera and the pelvic viscera appeared normal. The central nervous system was not examined.

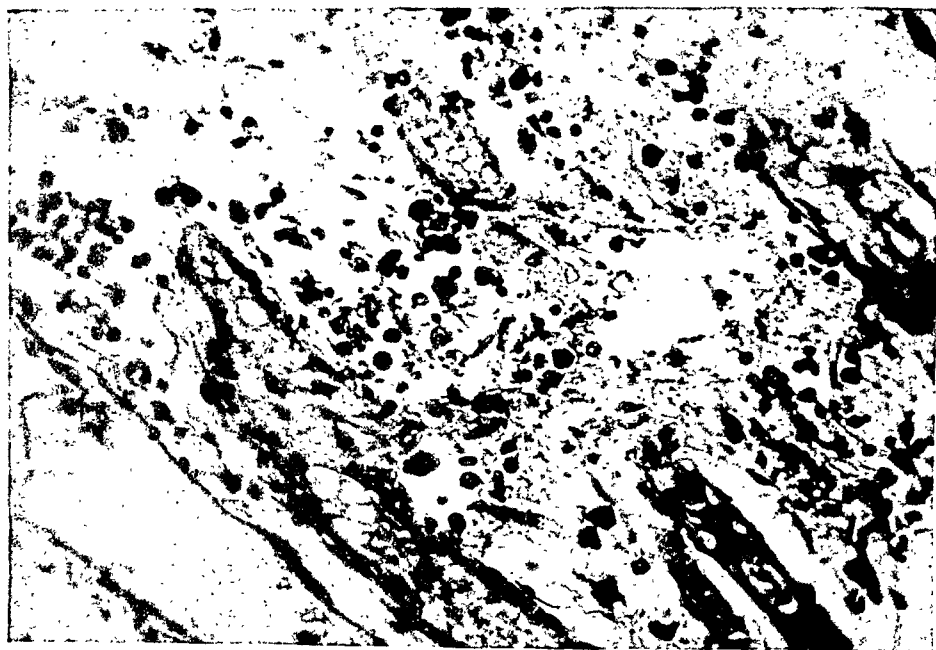


FIG. 3. Case 3. Photomicrograph of section of testicle exhibiting severe orchitis. $\times 350$.

Microscopic findings. In the heart, apart from mild irregular thickening of the mitral valve and mild arteriosclerosis, the interesting changes were limited to the myocardium (only the left ventricle was studied). The myocardium revealed interstitial edema, mild swelling of capillary endothelial cells and considerable perivascular infiltration by large mononuclear cells having broad cytoplasmic zones. A centrally degenerating, perivascular, mononuclear cell nodule completely filled an oil immersion field.

The sections of lungs exhibited a nonimpressive, patchy, intra-alveolar and bronchial exudate of neutrophils and large phagocytes, blood, mildly widened septa and some swelling of capillary and arteriolar endothelial cells. In the liver the Kupffer's cells were prominent and the sinusoids often contained an accumulation of large monocytes. The stroma about the portal triad was

edematous. Liver cells rarely showed fat replacement. The changes in the *spleen* were severe and largely confined to the pulp in which few erythrocytes, many large mononuclear cells, which were about five times the diameter of lymphocytes and had a basophilic cytoplasm, plasma cells and lymphocytes were seen. Macrophages with ingested erythrocytes were present in conspicuous numbers. The sinus endothelium, when recognized, was swollen. In the remnants of follicles, large mononuclears predominated. The *kidney* exhibited mild vascular nephrosclerosis, but, apart from the scarring, showed a few small interstitial nodules of lymphocytes and large pale mononuclear cells. The other organs studied microscopically, *viz.*, aorta, stomach and pancreas yielded no important information. Unfortunately no sections of brain, testis, or skin were available.

Case 3

Clinical data. C. W., a colored man, aged 56 years, was admitted to the Medical Service, U. S. Marine Hospital, New Orleans, Louisiana, on September 13, 1944, in an unconscious state. The history was obtained from his wife. With the exception of a nervous breakdown in 1919, he had been in good health until five days before admission, when he had a severe chill followed by fever. Chills and fever had continued up to the time of admission. There had been no recent known respiratory infection. Two days before admission he had complained of pain in his right shoulder, had a mild productive cough and severe night sweats. He had eaten and drunk very little since the onset of his illness. Urinary incontinence had been present for two days prior to admission.

On physical examination the patient was well developed, well nourished and mildly dyspneic. The skin was dry. No rash was noted, but its detection would have been difficult because of his dark skin. There was some diminution of breath sounds over the pulmonary bases and a few fine râles and scattered rhonchi were elicited over the left base. There was no evident enlargement of the heart. Auscultation appeared normal, but owing to labored respiration, the examination was not satisfactory. The blood pressure was 105/70. Palpation of the abdomen elicited mild muscle spasm but no other information. The genitalia appeared normal. There was no enlargement of lymph nodes.

The leukocyte count on admission was 18,300; hemoglobin was 14 gm. per cent; a differential count revealed neutrophils 81 per cent, (39 per cent band forms), lymphocytes 17 per cent and monocytes 2 per cent. The Wassermann and Kahn tests were positive. At the same time (fifth day of illness), agglutination tests for typhoid-paratyphoid group of organisms and *B. abortus* were negative. Proteus OX19 (Welch and Stuart antigen) agglutination was positive in dilution of 1:40. The urine was acid, specific gravity was 1.015, albumin content 300 mg. per cent, and centrifuged specimen yielded from 8 to 10 granular casts per high power field. A blood culture was sterile after seventy-two hours. A chest roentgenogram made on the day of admission revealed increased lung markings at the right base, which were interpreted as suggestive of bronchiectasis with a co-existent pneumonic process. The cerebrospinal fluid was clear and yielded no increase in cells or protein.

At the time of admission the rectal temperature was 106 F. and the pulse rate 120 per minute. As it was believed clinically that the patient had pneumonia, a total of 33 gm. of sulfadiazine was given. There appeared to be an initial improvement. The rectal temperature after twenty-four hours of treatment dropped to 102.5 F., but on the next day, the temperature rose to 104.3 F. and his condition appeared worse. In addition to sulfadiazine, he received 100,000 units of penicillin daily for four days. The rectal temperature rose to 107 F. and the pulse rate to 140 per minute. Death occurred six days after admission and about eleven days after the onset of illness.

Postmortem findings. The autopsy was performed by Dr. Joseph Gioia. The skin of the

arms and thighs exhibited questionable macules. There was a little pretibial and pedal edema. The peritoneal cavity contained about 200 cc. of clear fluid. The heart weighed 310 gm. No pericardial, myocardial, or endocardial abnormalities were noted. The right lung weighed 810 gm. The posterior part of the lower and middle lobes was rubbery in consistency and appeared edematous and congested, but there was no evidence of definite consolidation. The left lung weighed 520 gm. The lower lobe exhibited less edema and congestion than was seen on the right. The liver weighed 1730 gm. and showed no abnormalities. The spleen weighed 110 gm. and its pulp was dark red. The other viscera revealed no changes worthy of note. Apart from congestion, no lesions of the brain were noted.

Inasmuch as the gross examination did not reveal the cause of death, the clinical record was searched for clues which might suggest the course for additional investigation. The presence of Proteus OX19 agglutination titer of 1:40 on the fifth day of illness was noted; therefore, blood was obtained from the pelvic veins. As the Weil-Felix titer was shown to be 1:640, the death was reported as endemic typhus. Rickettsial complement-fixation test on the plasma (by Dr. Bengston) was reported positive for endemic typhus, 1:512 or higher, and positive for Rocky Mountain spotted fever, 1:16.

Microscopic findings. The epicardium, *myocardium* and endocardium exhibited edema and diffuse severe infiltration by many large mononuclear cells and plasma cells. Capillary endothelium was mildly swollen. Occasionally the infiltrate was perivascular in distribution.

In the *lung*, there was considerable congestion of septal capillaries with some extravasation of blood into alveoli. In some parts, bronchioles and adjacent alveoli were filled with sanguino-purulent exudate. A few septal capillaries appeared thrombosed. Very little interstitial infiltrate was seen. Some alveoli showed mildly swollen and hyperplastic lining cells.

Liver sections showed dilatation of small bile ducts. Generally the liver cells were swollen and somewhat vacuolated. There was a single focus of coagulation necrosis. The sinusoids were narrowed, contained little blood and a medium number of mononuclear cells, while Kupffer's cells were prominent.

The follicles of the *spleen* were of small size and the pulp was congested. Many aggregates of large mononuclear cells and plasma cells were in the pulp. Reticulo-endothelial cells were swollen.

The *kidney* exhibited large glomeruli and fibrinous material was seen rarely within their capillaries. The tubules were dilated. Scattered small infiltrates of mononuclear cells were seen in edematous interstitial tissues.

The tubules of the *testis* contained no spermatozoa. The germinal epithelium was hypoplastic. The interstitial tissues exhibited edema and a rather diffuse infiltration by plasma cells and large cells with pale nuclei and amphophilic cytoplasmic zones. In a few fields there was a little perivascular lymphocytic infiltration. Swelling of the capillary endothelium was conspicuous. In several small vessels there were subintimal blebs of fibrin and degenerating cells. A few endothelial cells contained minute, poorly stained basophil intracytoplasmic inclusions which were possible rickettsiae.

In the *rectus muscle* there was no necrosis. Capillary endothelial swelling was moderately advanced.

In the vertebral *bone marrow*, the young granulocytes were increased; there

were rather prominent shadowy bodies the size of megakaryocytes and fairly numerous large phagocytes exhibiting ingested nuclear debris and erythrocytes.

In two sections from the *skin* of one thigh there was an intense reaction in the deep corium which consisted of edema and perivascular infiltration by lymphocytes, plasma cells and large mononuclear cells with pale, eccentrically placed nuclei. Capillary endothelial cells were greatly swollen. Occasionally, it appeared that a capillary or arteriolar wall was entirely infiltrated by the inflammatory cells and obliteration had resulted. Several small vessels were occluded by thrombi. The inflammation extended to the subcutis. Sections of the skin from the forearm showed similar but less advanced changes. Sections of the *scrotum* revealed mild perivascular infiltration but changes were not as severe as those found in other areas of skin.

Sections of the aorta, thyroid, pancreas, prostate, adrenal and urinary bladder appeared normal.

In the *brain* congestion was severe. There was mild mononuclear cell infiltration of the leptomeninges of the cerebral cortex. The cerebellar meninges exhibited a distinct diffuse and perivascular infiltration by large mononuclear cells and swollen endothelial cells. In the corpus striatum, pons and in the cervical cord, mild perivascular mononuclear cell infiltration was seen. Mild monocytic meningeal infiltration was seen in the cervical cord. The pituitary appeared normal.

DISCUSSION

In general, the microscopic observations made in these three cases were similar. Acute interstitial myocarditis which was seen in each case probably was a major factor in the deaths. Damage due to cardiac muscle fibers was not evident. With exception of the two small nodules seen in Case 1, pulmonary changes were minimal. The microscopic appearances of the liver, spleen and lymph nodes could probably be duplicated by many infectious diseases. The obliterative thrombo-vasculitis and perivascular nodules of the skin resembled closely the lesions described in epidemic typhus. The severe interstitial orchitis occurred without recorded symptoms.

The brain changes consisted of edema, hyperemia, mild meningeal mononuclear cell infiltration and occasional perivascular mononuclear cell infiltration seen in meninges or within the brain. Endothelial cell swelling was not impressive. No "typhus nodules" were seen.

The splenomegaly (weight 1100 gm.) in Case 1 is far greater than that reported for various rickettsial diseases. From available literature the largest spleen reported for epidemic typhus¹¹ weighed 440 gm. and for Rocky Mountain spotted fever,⁶ 446 gm. In scrub typhus it has been described as twice normal size.

The severe jaundice in Case 1 was also unusual. Joseph,⁴ in a clinical study of 44 cases of typhus in New Orleans, observed jaundice in 1 case. It was seen in 2 of the 19 cases of fatal Rocky Mountain spotted fever studied by Lillie.⁶ Study of epidemic typhus in Naples by British investigators¹⁰ showed an inci-

dence of 4 per cent with jaundice. In addition to the splenomegaly and jaundice, the patient in Case 1 exhibited a rapidly progressive course, thrombocytopenia, severe anemia and leukopenia. Although the laboratory diagnosis of typhus appeared conclusive, the attending physicians believed that some other unidentified disease was present.

The slides of Case 1 were reviewed by the Staff of the Army Institute of Pathology, and by Dr. R. Philip Custer of Philadelphia and Dr. Henry Pinkerton of St. Louis, resident consultants for the Institute, who expressed the opinion that there had been some poorly defined form of bone marrow disease leading to myelosclerosis and splenomegaly prior to the attack of endemic typhus.

No other diseases to account for the fatal outcome were discovered in Cases 2 and 3. The possible harmful effect of sulfa drugs, which has been noted in other rickettsial diseases, was of course suggested by the fact that each of these was given sulfa therapy. In Case 2, the patient had been given twelve hours of treatment with sulfathiazole by his private physician and, after hospitalization, was given 8 gm. of sulfadiazine. As treatment for a probable pneumonia, the patient in Case 3 was given 33 gm. of sulfadiazine. In Case 1, atabrine and quinine were administered, but there was no record of the administration of a sulfa drug.

In Case 3, the diagnosis could not have been conclusive had not the disease been suspected at autopsy and suitable material taken.

SUMMARY

It appears that detailed autopsy reports have not been made on cases of endemic typhus from the United States. Pathologic studies of three cases, one from Mobile, Alabama and two from New Orleans, Louisiana are reported.

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OCCURRENCE OF A COMMON GROUP ANTIGEN AMONG *CANDIDA ALBICANS*, *SACCHAROMYCES CEREVISIAE* AND *HANSENULA ANOMALA**

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Although it has been known for many years that serologic cross reactions are demonstrable between members of the genus *Candida* and the group of naturally occurring true yeasts, there are few data concerning the reactions among organisms of established identity.

Epstein,² Fineman⁴ and Hines⁵ found cross agglutination reactions between *Candida* and true yeasts. Benham² likewise demonstrated a common antigen among *Candida*, *Hansenula* (Willia) and *Saccharomyces*. Almon and Stovall¹ pointed out that in these previous studies the identity of the yeasts was not clearly established and that in most instances high antibody titers were not obtained in experimentally immunized animals. The significance of such reports is consequently uncertain. The first study of this sort in which a number of yeastlike organisms was fully identified was reported by Almon and Stovall.¹ Employing organisms obtained from the American type culture collection and others that they identified, these authors by means of cross agglutination and agglutinin-absorption experiments found supportive evidence for the existence of a common group antigen among *Candida*, *Endomyces*, *Saccharomyces* and *Hansenula* (Willia).

Since confirmation of the work of Almon and Stovall¹ has not been reported as yet and since the results obtained by previous workers are difficult to evaluate, we have conducted cross agglutination and agglutinin-absorption experiments between *Candida* and other yeastlike organisms, particularly *Saccharomyces* and *Hansenula*. The present report deals with the results of this study.

MATERIALS AND METHODS

In Table 1 are listed the organisms employed and their sources. The species of *Candida* and *Geotrichum* were identified by study of the morphology and the biochemical reactions according to the method of Martin, Jones, Yao and Lee.⁶ The identity of the strain of *Candida albicans* was confirmed by Dr. Fred D. Weidman, Department of Research Dermatology, University of Pennsylvania.

C. albicans (K) fermented glucose and maltose with the production of both acid and gas. Sucrose was fermented with the production of acid but no gas. Lactose was not fermented. Young cultures were composed of oval, yeastlike forms, the colonies on Sabouraud's agar and on blood agar being round, creamy and butyrous, and measuring about 2 mm. in diameter. Four day old cultures on corn meal agar incubated under decreased oxygen tension showed typical

* Received for publication, July 12, 1947.

branching mycelia with chlamydospores. No pellicle was produced on Sabouraud's acid broth.

Candida tropicalis (ER7) fermented glucose, sucrose and maltose with the production of both acid and gas. Lactose was not fermented. Sabouraud's agar and blood agar cultures were composed of yeastlike forms, the colonies being round, creamy and butyrous, and measuring about 2 mm. in diameter. On corn meal agar there were well developed mycelia without chlamydospores. On acid broth a moderately heavy pellicle with numerous bubbles was produced.

TABLE 1
SOURCE OF ORGANISMS

ORGANISM	NUMBER	SOURCE
<i>Candida albicans</i>	K	Patient's sputum (bronchopulmonary moniliasis). Identified by Dr. Fred D. Weidman, Medical School, University of Pennsylvania
<i>Candida parakrusei</i>	ER3	Patient's skin (cutaneous moniliasis)
<i>Candida tropicalis</i>	ER7	Patient's skin (cutaneous moniliasis)
<i>Saccharomyces cerevisiae</i> (sporing variety)	A	Stock strains obtained by Department of Botany, University of Pennsylvania from Dr. Carl C. Lindegren, Department of Bacteriology and Immunology, Washington University School of Medicine and Henry Shaw School of Botany, Washington University, St. Louis, Missouri.
<i>S. cerevisiae</i> (non-sporing)	B	
<i>S. cerevisiae</i> (ellipsoid)	C	Stock strain obtained from Department of Botany, University of Pennsylvania.
<i>Hansenula anomala</i>	W1	Stock strain obtained from and identified by Dr. W. G. Hutchinson, Department of Botany, University of Pennsylvania.
<i>Geotrichum lactis</i>	5417	Patient's sputum (chronic bronchitis)
<i>Cryptococcus neoformans</i>	T1	Patient's spinal fluid (torulosis) obtained from Dr. Fred D. Weidman.

Candida parakrusei (ER3) fermented glucose with the production of acid and gas. After prolonged incubation only a slight amount of acid appeared in the sucrose and maltose tubes. Lactose was not fermented. On blood agar small, gray, low, irregular colonies composed of yeastlike cells were produced. On corn meal agar typical branched mycelia with lateral conidia were formed. No pellicle was produced in acid broth.

Geotrichum lactis (5417) fermented glucose with the production of acid only. Lactose, sucrose and maltose were not fermented. The first subculture on blood agar gave colonies measuring about 2 mm. in diameter with a limited amount of

aerial mycelia. Subsequent subcultures on Sabouraud's agar gave rugose, creamy colonies with widely spreading mycelial fringe which extended into the agar. Corn meal agar cultures showed septate branching mycelia composed of arthrospores. In two day old preparations the arthrospores were very abundant and dispersed, appearing as large bacillary and occasionally as large coccoid forms.

Immune serums were prepared by the intravenous inoculation of rabbits weighing approximately 2 Kg. with the respective organisms according to the method of Benham.² At the start of the experiment, the rabbits had no demonstrable agglutinins for the homologous organisms. The organisms were grown on Sabouraud's glucose agar for not more than twenty-four hours, were suspended in physiologic salt solution, and were then killed by heating at 60 C. for one hour.

TABLE 2

AGGLUTINATION REACTIONS OF VARIOUS ANTISERUMS WITH HOMOLOGOUS ORGANISMS AND *Candida albicans*

ANTISERUM	ANTISERUM NUMBER	TITER OF HOMOLOGOUS ORGANISMS	TITER OF <i>C. Albicans</i> ORGANISMS
<i>Cryptococcus neoformans</i>	4	0	0
<i>C. neoformans</i>	5	0	0
<i>Geotrichum lactis</i>	11	1:2560	0
<i>G. lactis</i>	12	1:2560	0
<i>Saccharomyces cerevisiae</i> "A".....	13	1:10240	1:640
<i>S. cerevisiae</i> "A".....	14	1:10240	1:320
<i>S. cerevisiae</i> "B".....	15	1:10240	1:640
<i>S. cerevisiae</i> "B".....	16	1:10240	1:640
<i>S. cerevisiae</i> "C".....	17	1:5120	1:640
<i>S. cerevisiae</i> "C".....	18	1:5120	1:640
<i>Hansenula anomala</i>	8	1:2560	1:1280
<i>H. anomala</i>	9	1:2560	1:1280

Blood for testing was drawn from the ear vein ten days after completion of the inoculations, except when otherwise indicated.

Serum agglutinins were titrated in studies of cross agglutination and in agglutinin absorption tests by the method we have previously described.⁷ The final dilution of antigen employed in all cases was 1:3000. Serial dilutions of serum were made from 1:5 to a final dilution of 1:10,240.

Agglutinins were absorbed by adding approximately 10 volumes of a 1:2.5 dilution of serum to 1 volume of packed cells. The mixture was then incubated in a water bath at 37 C. for two hours. The suspension was centrifuged and the supernatant was absorbed again with fresh antigen. If agglutination was observed, upon completion of the second absorption, the absorption was repeated until none was visible. As a control for the completion of absorption, the supernatant, constituting the absorbed serum, was titrated against the absorbing antigen for the presence of agglutinins. Unabsorbed serum in all cases was incubated together with absorption mixtures to furnish a controlled standard of reference.

RESULTS

In Table 2 it will be seen that inoculation of the rabbit with *Cryptococcus neoformans* (*Torula histolytica*) did not result in the formation of agglutinins for the homologous organism or for *C. albicans*. All of the other organisms, however, were highly antigenic in the rabbit. Except for *Geotrichum*, the antiserums for these organisms also strongly agglutinated *C. albicans*. On the other hand, in Table 3, it is seen that seven antiserums for one strain of *C. albicans* agglutinated *Saccharomyces cerevisiae* in substantially the same titer as with the homologous organism. Table 4 indicates that the antiserums for *S. cerevisiae* and *Hansenula*

TABLE 3
AGGLUTINATION REACTIONS OF *Candida albicans* ANTISERUMS WITH *C. albicans*
AND *Saccharomyces cerevisiae*

<i>C. albicans</i> ANTISERUMS	TITER OF <i>C. albicans</i> ORGANISMS	TITER OF <i>S. cerevisiae</i> "A" ORGANISMS
#23	1:1280	1:640
#24	1:640	1:1280
#28	1:2560	1:1280
#32	1:1280	1:1280
#33*	1:640	1:320
#19*	1:160	1:80
#25*	1:320	1:640

* Blood was drawn one month following completion of final course of inoculations.

TABLE 4
AGGLUTINATION REACTIONS OF *Hansenula anomala* AND *Saccharomyces cerevisiae*
ANTISERUMS WITH VARIOUS ORGANISMS

ORGANISMS	ANTISERUM	
	Titer of <i>H. anomala</i> #8	Titer of <i>S. cerevisiae</i> "A" 13
<i>S. cerevisiae</i>	1:1280	1:5120
<i>H. anomala</i>	1:1280	1:160
<i>Candida albicans</i> ..	1:640	1:320
<i>Candida parakrusei</i>	1:160	1:80
<i>Candida tropicalis</i>	1:80	1:20

anomala cross agglutinated the heterologous organism, and the antiserums for both cross agglutinated *C. parakrusei* and *C. tropicalis* in addition to *C. albicans*.

Since each of the three strains of *S. cerevisiae* completely absorbed not only the agglutinins of the homologous serums but also those of the other two serums, these organisms were considered antigenically identical and only strain "A" was employed in the following absorption tests.

In Table 5, it is shown that both *C. albicans* and *S. cerevisiae*, respectively, absorbed the greater part of the agglutinins for *H. anomala*. The absorption of *S. cerevisiae* antiserum with either *C. albicans* or *H. anomala*, however, did not re-

duce the original titer of the serum for the homologous organisms. A like result was obtained when *C. albicans* antiserum was absorbed with *S. cerevisiae* and *H. anomala*, respectively.

After absorption of antiserum for *C. albicans* with *S. cerevisiae*, the absorbed serum did not agglutinate *H. anomala*. Likewise, after absorption with *H. anomala*, *C. albicans* antiserum did not agglutinate *S. cerevisiae*.

DISCUSSION

The cross agglutination and agglutinin-absorption experiments indicate the existence of a common antigen which is shared by the three species of *Candida* and each of the species of true yeasts tested. In this regard the work of Almon and Stovall¹ has been confirmed. In Table 4 it is shown that our strain of *C.*

TABLE 5
AGGLUTINATION REACTIONS OF ANTISERUMS WITH VARIOUS ORGANISMS FOLLOWING
COMPLETE ABSORPTION WITH INDICATED ORGANISMS

ANTISERUM	ABSORBING ORGANISM	SUBSEQUENT AGGLUTINATION	
		Organism tested	Titer
<i>Hansenula anomala</i> #9	<i>C. albicans</i>	<i>H. anomala</i> #9	1:20
<i>H. anomala</i> #9	<i>S. cerevisiae</i> "A"	<i>H. anomala</i> #9	1:40
<i>H. anomala</i> #9	None (control)	<i>H. anomala</i> #9	1:640
<i>Saccharomyces cerevisiae</i> "A" #13	<i>C. albicans</i>	<i>S. cerevisiae</i> "A" #13	1:5120
<i>S. cerevisiae</i> "A" #13	<i>H. anomala</i>	<i>S. cerevisiae</i> "A" #13	1:5120
<i>S. cerevisiae</i> "A" #13	None (control)	<i>S. cerevisiae</i> "A" #13	1:5120
<i>Candida albicans</i> #3	<i>S. cerevisiae</i>	<i>C. albicans</i> #3	1:1280
<i>C. albicans</i> #3	<i>H. anomala</i>	<i>C. albicans</i> #3	1:1280
<i>C. albicans</i> #3	None (control)	<i>C. albicans</i> #3	1:1280
<i>C. albicans</i> #2	<i>S. cerevisiae</i>	<i>H. anomala</i>	0
<i>C. albicans</i> #2	<i>H. anomala</i>	<i>S. cerevisiae</i>	0
<i>C. albicans</i> #2	None (control)	<i>C. albicans</i>	1:1280

tropicalis was not agglutinated in as high a titer as our strain of *C. albicans* by antisera for *Hansenula* and *Saccharomyces*. So far as this evidence goes, our results are in disagreement with those of Almon and Stovall¹ that these species are serologically identical. Our strain of *C. neoformans* produced no detectable agglutinins in the rabbit. Evidently the antigenic structure of *Geotrichum* is distinct from *Candida*, since the *Geotrichum* antisera did not agglutinate these organisms.

Estimates of the approximate relative proportions between common and species-specific agglutinins in the rabbit suggests that *C. albicans* and *S. cerevisiae* antisera each contain predominantly effective homologous species-specific agglutinins, but that *H. anomala* antiserum contains predominantly effective common agglutinins. These results likewise confirm the findings of Almon and Stovall.¹

SUMMARY

By means of cross agglutination and agglutinin-absorption reactions, a group antigen common to *Candida* (*Monilia*) *albicans*, *Saccharomyces cerevisiae*, and *Hansenula* (*Willia*) *anomala* is demonstrated. Immune antisera for *Candida* and *Saccharomyces* contain predominantly effective species agglutinins; antisera for *H. (Willia) anomala* contain predominantly effective group agglutinins. The previous work of Almon and Stovall¹ is confirmed.

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OCCURRENCE OF SERUM AGGLUTININS FOR *CANDIDA ALBICANS* AND *SACCHAROMYCES CEREVISIAE* IN A HOSPITAL POPULATION*

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The occurrence of serum agglutinins for *Candida* (*Monilia*) *albicans* in man has not been widely studied and a comparison of the titers for this organism with those for *Saccharomyces* has not been reported. In chronic infections thought to be caused or aggravated by *Candida*, serum agglutinin titers have been studied in individual cases. Thus titers have been reported in cases of chronic bronchopulmonary disease in which *Candida* (*Monilia*) was constantly present in the sputum, by Kurotechkin and Chu,⁹ Bakst, Hazard and Foley,² Norris,¹¹ Koerth, Donaldson and McCorkle,⁸ and Pimentel Imbert;¹² in cutaneous moniliasis by Hopkins;⁶ and in vegetative endocarditis with systemic mycosis by Joachim and Polayes.⁷ The titers ranged from 0 to 1:640. On the other hand, in a group of 1150 presumably normal individuals, Todd¹⁵ reported the presence of serum agglutinins for *C. (Monilia) albicans* in titers of 1:10 or higher in 259, or 22.5 per cent. The incidence of detectable agglutinins in women was 30.4 per cent compared with 15.7 per cent in men. The greatest incidence was in the group having titers of 1:40. Twenty-six serums gave a titer of 1:160, 8 a titer of 1:320, and 1 a titer of 1:640. In all but 1 of the 26 persons, *C. albicans* was cultured from the mouth or throat. For this reason, Todd concluded that a definite relationship existed between the presence of organisms in the body and the high antibody titers. Among 26 individuals showing no clinical evidence of *Candida* infection, Bakst, Hazard and Foley² found that 17 had agglutinin titers of from 1:5 to 1:80. Recently, however, Fuentes and Guarton,⁵ in a study of the serums of 1002 unselected patients found only 2 who showed agglutinins in titers of 1:20 or higher; one in a titer of 1:20 and the other in a titer of 1:80. In 10 patients having vaginitis thought to be caused by *Candida*, serum agglutinins for this organism were not detected. Although their findings were quite different from those of Todd, the authors concluded that when detectable serum agglutinins for *Monilia* are present, clinically important moniliasis is likely to be present.

From the evidence cited, it is apparent that more data will be required in patients not suspected of harboring *Candida* as well as in patients in whom *Candida* is thought to be clinically pathogenic, before the presence or absence of serum agglutinins for this organism can be evaluated. For this reason, the titers of serum agglutinins for *C. albicans* have been estimated in a group of serums submitted for the serologic diagnosis of syphilis. Since it is known that cross agglutination reactions occur in experimentally immunized animals, not only between species of *Candida* but also between *Candida* and other yeastlike fungi

* Received for publication, July 12, 1947.

(Fineman,⁴ Benham,³ Almon and Stovall,¹ Martin,¹⁰ and Rawson and Norris,¹⁴) the agglutinin titers of more than half the serums for a stock strain of *Saccharomyces cerevisiae* were determined simultaneously.

MATERIALS AND METHODS

The serums of 469 persons submitted to the laboratory for routine serologic tests for syphilis were employed. Since a correlation of serums having detectable agglutinin titers for *Candida* with positive serologic tests for syphilis was contemplated, more serums giving positive Kolmer and Kline tests were selected than would have occurred by random sampling. In several instances, the serums

TABLE 1

THE INCIDENCE OF AGGLUTININS FOR *Candida albicans* ACCORDING TO TITER
AMONG 469 PATIENTS

	TITER							
	0	1:5	1:10	1:20	1:40	1:80	1:160	1:320
Number of patients	169	83	92	68	29	21	4	3

TABLE 2

TITER OF AGGLUTININS IN 464 SERUMS, ACCORDING TO SEX OF PATIENT

Children are listed separately. The numerals indicate the number of persons in each group.

TYPE OF PATIENT	TITER OF AGGLUTININS FOR <i>Candida albicans</i>								TOTAL NUMBER OF PATIENTS
	0	1:5	1:10	1:20	1:40	1:80	1:160	1:320	
Woman adults	109	54	63	54	25	11	4	3	323
Men adults	50	27	24	14	4	9	0	0	128
Children	6	2	5	0	0	0	0	0	13
Totals	165	83	92	68	29	20	4	3	464

of patients known clinically to harbor *Candida* for long periods of time were excluded. Otherwise, there was no effort at selection.

The strains of *C. (Monilia) albicans* and *S. cerevisiae* employed were those used in our previous study¹⁴ and their characteristics will not be detailed again. The antigens were prepared for use and the agglutination tests were performed according to the method we have previously reported.¹² The serums had been inactivated prior to testing. No difficulty with spontaneous agglutination of the antigen was experienced in controls of physiologic solution of sodium chloride or in negative serum.

RESULTS

In Table 1, it will be seen that 300 of 469 serums gave agglutinin titers for *C. albicans* of 1:5 or greater, an incidence of 64 per cent, and 216 gave titers of 1:10

or greater, an incidence of 46 per cent. The observed incidence is thus twice that reported by Todd,¹⁵ who found that only 22.5 per cent of serums gave titers of 1:10 or higher and is obviously greatly in excess of that reported by Fuentes and Guarton,⁵ who found only 2 serums of 1002 with titers of 1:20 or higher.

Of the total number of 469 serums, the age and sex were known in 464. In Table 2, children under 13 years are listed separately. The remainder are grouped according to sex. A higher incidence of serum agglutinins is observed in females than in males, but the difference in incidence is not so great as that reported by Todd.¹⁵ The difference is greatest when agglutinins at titers of 1:20

Titer of agglutinins for <i>Saccharomyces cerevisiae</i>	1:80	0	0	0	0	1	0	0	0
	1:40	0	0	4	0	1	3	0	0
	1:20	0	2	7	5	1	2	0	0
	1:10	6	10	7	9	4	0	0	0
	1:5	18	11	11	9	1	2	1	0
	0	70	23	21	14	5	0	2	1
		0	1:5	1:10	1:20	1:40	1:80	1:160	1:320
		Titer of agglutinins for <i>Candida albicans</i>							

FIG. 1. The titers of serums for *S. cerevisiae* are plotted on the ordinate and for *C. albicans* on the abscissa in each case. The numbers in the squares indicate the number of persons in each group.

or higher are compared: women positive in 30 per cent, men in 21 per cent. This ratio is not significantly different from the 2:1 ratio reported by Todd, but on the other hand, the figures in themselves do not establish statistically a preponderance of agglutinins among women.

When the presence or absence of detectable agglutinin titers for both *C. albicans* and *S. cerevisiae* are compared (Fig. 1) for the serums of 251 patients, it will be seen that 181 serums, or 72 per cent, had measurable titers to one or both organisms. One hundred and fifteen had positive titers for *S. cerevisiae* and 157 for *Candida*. Of these, 91 serums agglutinated both organisms. On the other

hand, only 70, or 28 per cent, of the serums had no detectable agglutinins to either *Candida* or *Saccharomyces*. When these results are analyzed it is striking to observe that those serums which have no agglutinins for one organism generally have no agglutinins for the other organism. Conversely, when a serum has agglutinins for one organism, there is a highly significant tendency for that serum also to agglutinate the second organism. In those serums which agglutinate both organisms, however, there is no correlation between the height of the individual titers for one organism as compared with the titer for the other.

Correlation of Presence of Agglutinins for C. albicans with Positive Reactions for Syphilis

In the course of the study, several specimens also gave positive serologic tests for syphilis. The question arose, therefore, whether any correlation might exist between positive tests for syphilis and high titers for *C. albicans*. If a correlation should exist, the rôle of immunity to *C. albicans* as a possible cause of false positive serologic tests for syphilis would require further investigation. No data on this subject were found in the literature.

The serologic tests for syphilis employed were the qualitative Kolmer complement-fixation test and the diagnostic Kline flocculation test. Of the 469 serums in which the presence or absence of agglutinins was determined, the results of the Kolmer and Kline tests in 458 were considered satisfactory for comparison. The eleven tests remaining were not included since the serums were found to be anticomplementary when the Kolmer tests were read. More serums with positive serologic tests for syphilis were selected than would have been obtained by random sampling alone.

In Figure 2, the readings of Kolmer and Kline tests are plotted together along the ordinate against the readings of the *Candida* agglutination tests along the abscissa. When the figures are analyzed, there is no more coincidence between positive Kolmer and Kline tests and the presence of serum agglutinins for *C. albicans* than would be expected from chance distribution. Consequently a more detailed study including quantitative serologic tests for syphilis was not undertaken.

COMMENT

From the data presented, the incidence of detectable serum agglutinins for *C. albicans* in a group of hospital patients is higher than has previously been reported. Since no difficulty was experienced with spontaneous agglutination of the antigen, which was prepared from growths of not more than twenty-four hours' duration on Sabouraud's agar, it is believed that the results were not caused by technical error. It is possible that the present strain of *C. albicans* was agglutinated by human serums more readily than were the strains employed by others, especially that of Fuentes and Guarton.⁵ It is also possible that in the particular hospital population studied, the incidence of exposure to *Candida* was greater than in other localities. An explanation as to the difference in sex incidence reported by Todd¹⁵ as compared with our results is not apparent.

The tendency for serums to show no agglutinins for either *Candida* or *Saccharomyces* or conversely to show agglutinins for both of them is to be expected

Readings of Kolmer and Kline Tests	Kolmer	Kline							
		4	8	4	7	2	0	0	0
4		3	2	0	1	1	0	0	0
		2	5	0	1	0	0	0	0
		1	1	1	1	0	0	0	0
		0 or \pm	0	0	0	0	0	0	0
		4	3	0	0	1	0	0	0
3		3	5	2	2	0	0	0	0
		2	1	1	1	0	0	0	0
		1	0	0	0	0	0	0	0
		0 or \pm	0	0	0	0	0	0	0
		4	0	2	0	1	0	0	0
2		3	0	0	0	1	0	0	0
		2	2	1	2	0	0	0	0
		1	1	0	0	1	0	0	0
		0 or \pm	0	0	0	0	0	0	0
		4	0	0	1	1	0	0	0
1		3	2	1	1	0	0	1	0
		2	0	0	0	0	0	0	0
		1	1	0	0	1	0	0	0
		0 or \pm	2	0	0	1	0	0	0
		4	2	0	1	0	0	0	0
0 or \pm		3	3	1	0	1	0	0	0
		2	4	6	7	4	2	0	1
		1	7	5	3	2	3	1	0
		0 or \pm	116	52	68	50	22	18	4
									2
		0	1:5	1:10	1:20	1:40	1:80	1:160	1:320
		Titers for <i>Candida albicans</i>							

FIG. 2. The readings of the qualitative Kolmer and diagnostic Kline tests are plotted on the ordinate and the titers of serums for *Candida albicans* on the abscissa for each case. The numerals indicate the number of cases in each group.

because of the group antigen present in both organisms. The lack of correlation between the height of the agglutinin titer for *Candida* and *Saccharomyces* may

actually be due to immunization in individual cases against the organism yielding the higher titer. Since individuals are likely to be exposed to both organisms, however, this explanation is by no means certain. Among this group of serums, furthermore, the occurrence of agglutinins for other fungi was not investigated.

In view of these possibilities, the clinical records of patients having serums with high titers for either *Candida* or *Saccharomyces* or both were reviewed. None was suspected clinically of harboring *Candida* or any other yeastlike organism. The diagnoses were scattered over a wide range of medical conditions and a factor common to this group was not discovered.

Because of the variation in incidence of serum agglutinins for *C. albicans* so far reported and because of the known cross agglutination reaction between *Candida* and other yeastlike fungi, it is concluded that the presence of serum agglutinins for *Candida* in an individual is not specific evidence that *Candida* is harbored in the tissues of the patient.

SUMMARY

The agglutinins for *Candida* (*Monilia*) *albicans* were titrated in 469 serums submitted to the laboratory for routine serologic tests for syphilis. Agglutinin titers for *Saccharomyces* were determined simultaneously in 251 serums. The incidence of detectable agglutinins was greater for *Candida* than that reported by other workers and, contrary to previous reports, the number of women with demonstrable agglutinins was not significantly preponderant. There was a significant trend for serums to have no agglutinins for either *Candida* or *Saccharomyces* or to have detectable agglutinins for both organisms. Because of the high incidence of serum agglutinins for *Candida* in a hospital population and because of the known cross agglutination reactions between *Candida* and other yeastlike fungi, the agglutination test should be used only as an adjunct in clinical diagnosis.

Qualitative Kolmer complement-fixation tests and Kline diagnostic flocculation tests for syphilis and an estimation of the serum agglutinin titer for *C. albicans* were performed on 458 of these serums. When the results of individual tests were charted, there was no more relation between a positive Kolmer and Kline test and the presence of detectable agglutinins for *C. albicans* than would be expected by the laws of chance.

Acknowledgment. The authors wish to thank Dr. J. Harold Austin for his aid in the analysis of the results.

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CHARACTERISTIC URINARY FINDINGS IN VISCERAL ANGIITIS (PERIARTERITIS NODOSA, LUPUS ERYTHEMATOSUS)*

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Renal damage is frequently seen in "visceral angiitis", a term suggested to cover that group of diseases for which a common denominator has long been sought.^{2, 5, 8} Although detailed descriptions of the histopathology have been published,^{3, 4, 5, 9} only one report, that of Krupp,⁶ emphasizes characteristic urinary findings. Krupp studied 21 cases of visceral angiitis and found a characteristic urinary sediment in 14. This consisted of the simultaneous presence of elements usually characteristic of the early stage of nephritis (erythrocytes and erythrocytic casts) and those usually seen in the chronic stage (broad casts, waxy casts, fatty casts and "oval fat bodies").

I have recently had occasion to study two patients with periarteritis nodosa and one with acute disseminated lupus erythematosus. The diagnoses in these cases were proved by biopsy or autopsy, a complete clinical report being presented elsewhere.⁷ Freshly voided or catheterized first morning urine specimens were examined. It was impractical to study the sediment by the method of Addis,¹ as Krupp did, since these patients were very ill and it did not seem justifiable to subject them to a prolonged period of reduced fluid intake. Specimens from all three patients showed proteinuria and the unusual sediment previously described (Table 1). This urinary picture has been described so seldom in acute glomerulonephritis that when it is encountered a presumptive diagnosis of visceral angiitis seems justified.

In Case 1 the patient had been hospitalized for an allergic dermatitis, at which time the urine was entirely normal. The onset of acute panarteritis was sudden and dramatic, and within one week the urinary findings were distinctly abnormal. As this patient recovered, daily studies revealed improvement in the proteinuria and in the sediment. In Case 2 the disease had probably been present in the patient for about one year, but the acute exacerbation preceding death lasted about one month. It is noteworthy that the urine previously showed only small quantities of protein and a few hyaline casts. The patient in Case 3 had typical acute lupus erythematosus, apparently becoming subacute, and again there was gradual improvement in the urinary sediment as the acute phase subsided.

A study of six cases of subacute lupus erythematosus supports the evidence that this characteristic sediment is present only during the acute phase. In the subacute phase proteinuria is slight and hematuria and casts are absent. The disease in Krupp's cases varied in duration from one to thirty-six months, but he also noted that a change in the urinary findings paralleled the clinical improvement. Obviously a large number of cases must be studied from this standpoint

* Received for publication, June 19, 1947.

before a definite statement can be made of the relation of the nature of the sediment to the duration of the disease. Admittedly more quantitative results can be obtained if Addis counts are done and they should be performed whenever

TABLE 1

SUMMARY OF URINARY FINDINGS IN TWO PATIENTS WITH PERIARTERITIS NODOSA AND ONE PATIENT WITH LUPUS ERYTHEMATOSUS

CASE NUMBER	AGE, COLOR AND SEX	DIAGNOSIS	DURATION OF DISEASE	URINARY FINDINGS	
				Albumin in mg./100 ml.	Sediment
1	40 W M	Acute periarteritis nodosa, confirmed by biopsy	1 week	400	Many leukocytes, erythrocytes, erythrocytic casts, waxy casts, casts with fatty vacuoles and broad casts
			3 months later, disease quiescent	60	Very occasional hyaline casts
2	67 W M	Periarteritis nodosa, confirmed by autopsy	? 1 year	200	Many leukocytes, erythrocytes, erythrocytic casts, hyaline casts, granular casts and casts with fatty vacuoles
3	19 W F	Acute lupus erythematosus disseminatus, confirmed by biopsy	2 weeks	750	Many leukocytes and erythrocytes, many erythrocytic casts and granular casts; few hyaline, waxy and vacuolated casts
			6 weeks later, disease in subacute phase	60	Occasional leukocytes and erythrocytes, very occasional erythrocytic casts and hyaline casts

possible. Since it is possible to recognize the unusual sediment on routine urinalysis, however, its value as a diagnostic aid is thereby enhanced.

SUMMARY

A previous report of characteristic urinary findings in visceral angiitis is supported by three additional cases, in which there was the simultaneous presence

in the urine of elements usually characteristic of various stages of nephritis. The picture seems to be characteristic during the acute phase of the disease, and to disappear with remission.

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MICROFILARIAL GRANULOMAS, ELEPHANTIASIS AND ADENOSIS OF THE BREAST*

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In filariasis, especially in infections with *Wuchereria bancrofti*, the majority of the lesions are caused by adult worms. Up to recent times the microfilariae were considered harmless,⁷ although it was known that degenerating microfilariae, especially in hydroceles, might cause tissue changes.¹⁰ Meyers and Kouwenaar⁸ recently demonstrated *Microfilaria malayi* in eosinophilic abscesses in the inguinal lymph nodes of patients with tropical eosinophilia. Bonne¹ described a marked eosinophilia of the spleen associated with microfilarial infestation of this organ, the terminal ends of the worms being surrounded by peculiar giant cells. Van der Sar and Hartz¹¹ reported an almost identical case. Dhayagude and Amin² reported 11 cases of microfilarial granuloma of the spleen with marked eosinophilia in the granulomas. In a subsequent paper, Dhayagude² described 12 additional cases of these granulomas. Van der Sar and Hartz^{11, 12} found microfilariae (probably *Microfilaria bancrofti*) in eosinophilic infiltrates and abscesses in the axillary lymph nodes in cases of tropical eosinophilia. They expressed the opinion that microfilariae are among the causative agents of tropical eosinophilia.

These reports show that the microfilariae are not as harmless as formerly supposed and, in view of the increasing interest in the lesions provoked by these parasites, it seems proper to report a case in which microfilarial granulomas were found in the breast as a complication of elephantiasis of the breast. As far as we can ascertain, this is the first such case reported in the literature.

REPORT OF CASE

Clinical Data

A colored woman, 29 years old, had complained for a few days before her admission of pain in the left breast. She had not observed any discharge from the nipple. Her left breast had always been a little larger than the right. The patient had two children, 5 and 6 years old, whom she had nursed herself. Menstruation had been somewhat irregular. Examination showed an obese woman with enormous breasts, the left breast being a little larger than the right. There was some retraction of the left nipple, whereas the right nipple was normal. The subareolar tissue of the left breast was firmer; this firmer area could not be well delimited. The overlying skin could not be moved or lifted. There was some indication of "orange peel" skin. Surgical exploration was done and a moderate amount of whitish, serous tissue was removed. It was observed during operation that the ducts were distended and contained putty-like material. A blood count, performed three weeks after operation, showed the presence of 6 per cent eosinophilic leukocytes. In blood taken at night, microfilariae were found.

* Received for publication, July 10, 1947.

Microscopic Examination

The breast tissue showed numerous lobules lying in dense connective tissue, containing few cells. In many places the connective tissue was edematous. The acini were distended and filled with an acidophilic, homogenous substance. Many small ducts showed irregular proliferation of the epithelium; other ductuli were distended by inspissated material. There was also marked dilatation of the larger ducts which contained either colloid-like material or a more granular substance with rounded vacuoles, desquamated epithelium and vacuolated histiocytes. There were a few cysts and tubules with so-called pale epithelium. Several larger ducts were surrounded by infiltrates composed mainly of lymphocytes and plasma cells. The lymph vessels were markedly distended and very conspicuous. They often contained a pale-staining, homogenous substance or small groups of lymphocytes. In other lymph vessels there were smaller or larger accumulations of closely packed histiocytes with an admixture of lymphocytes, which sometimes filled a large part of the lumen. In the connective tissue sometimes closely adjacent to a lymph vessel, there were small nodules composed of histiocytes and a few lymphocytes. They each contained a typical microfilaria. There was no fibrosis of these nodules. No eosinophilic leukocytes were found in the connective tissue or in the blood vessels. A few small blood vessels were surrounded by sheaths of lymphocytes; otherwise they did not show pathologic changes. There was relatively little fatty tissue; the small lobules of adipose tissue were separated by dense fibrous septa and there was more connective tissue than usual in the lobules.

DISCUSSION

The changes of the acini and ducts together with the presence of small cysts and tubules with the so-called pale epithelium are typical of beginning adenosis. It is not so easy, however, to find an explanation for the enormous enlargement of the breasts, especially since the patient did not remember when she first noticed the enlargement. Therefore, the presence of a previously existing virginal or postlactational hypertrophy cannot be excluded. Our case, however, presents features which are not found in the usual types of mammary hypertrophy,^{4, 9} *e.g.*, the edema, the marked dilatation of the lymph vessels and the granulomatous endolymphangitis. These changes are typical of filariasis,⁵ especially when accompanied by lymphatic obstruction. As the patient was proved to be suffering from filariasis, we think the conclusion is warranted that there was filarial elephantiasis of the breast, perhaps superimposed on some type of mammary hypertrophy.

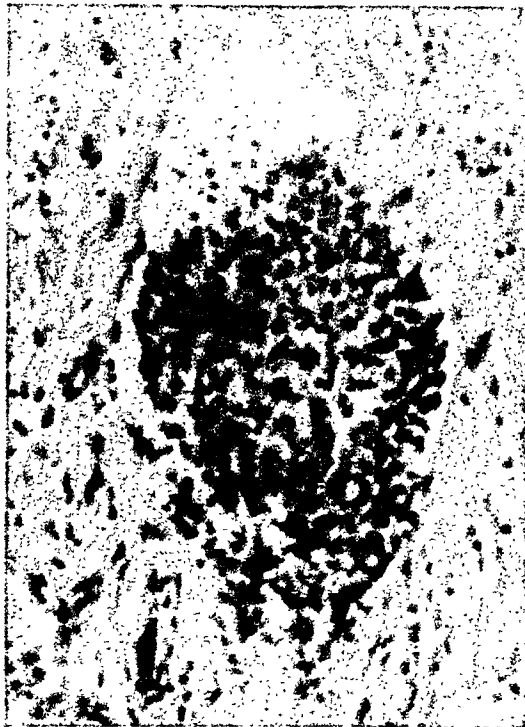
In some cases of mammary hypertrophy, the breasts may attain an enormous size^{4, 9} and such hypertrophy may have been present in cases which have been

FIG. 1. Granulomatous endolymphangitis. There is edema of the connective tissue. $\times 320$.

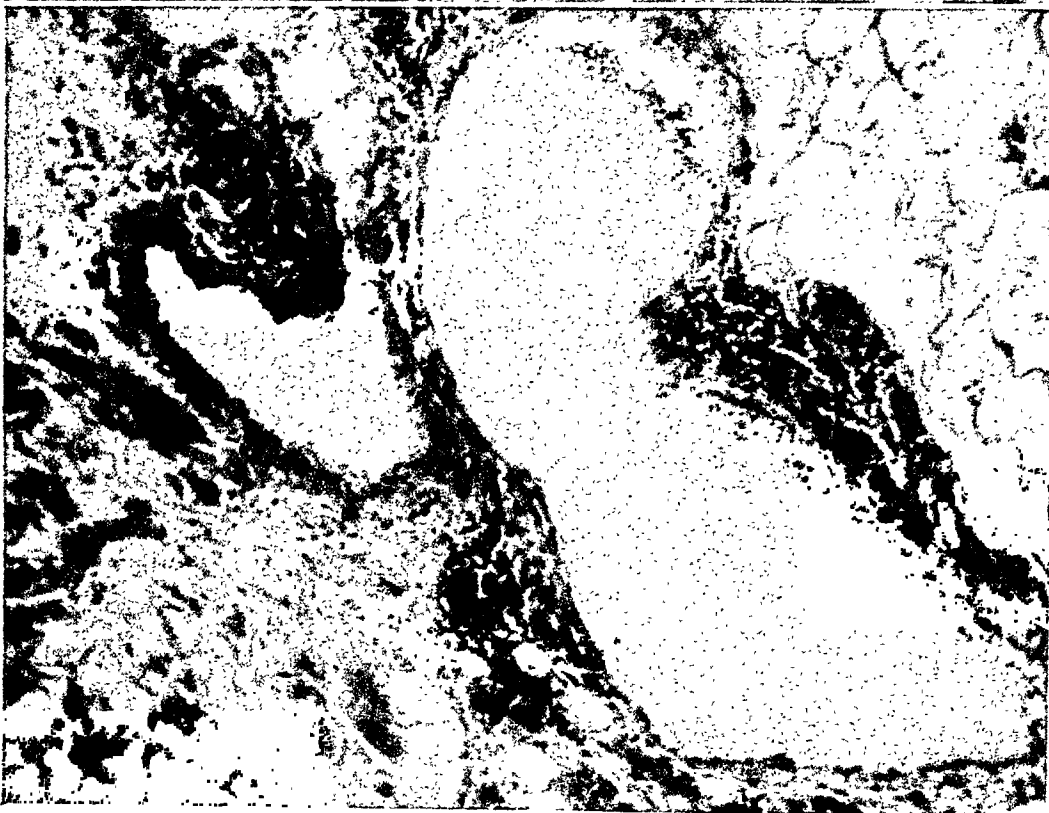
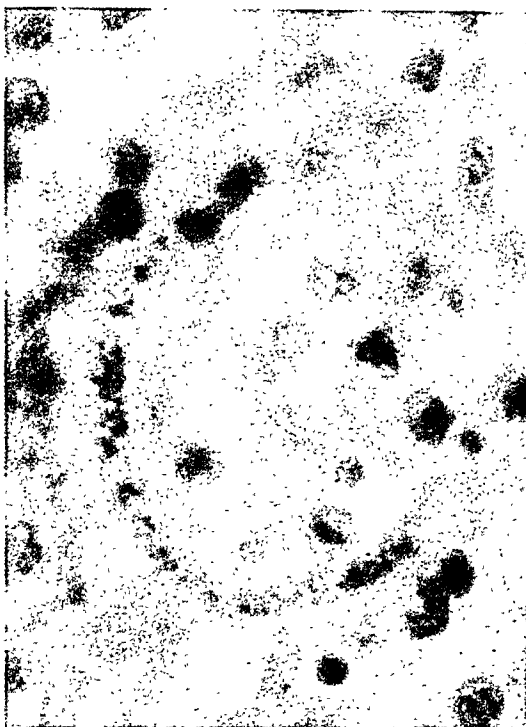
FIG. 2. Microfilaria lying in a small granuloma. $\times 1400$.

FIG. 3. Dilated lymph vessels containing colloid-like material and a few lymphocytes. $\times 160$.

1



2



3

labeled elephantiasis of the breast, especially since this affection is rare. This, however, can only be decided by histologic examination, which, in cases of elephantiasis, should show the presence of edema, increase of the connective tissue and especially dilatation of the lymph vessels. The eventual finding of granulomatous endolymphangitis, as in our case, will facilitate establishment of a diagnosis. In tropical countries, where elephantiasis of the breast is most likely to occur, it may be difficult to obtain permission for removal of tissue for biopsy, so that in most cases a final decision cannot be reached.

The microfilariae that had in some way lodged in the stroma of the breast, had provoked the formation of small granulomas. Although fibrotic changes in the granulomas were completely absent, there were no eosinophilic leukocytes. This is in marked contrast with the cases of Dhayagude and Amin,³ of Bonne¹ and of van der Sar and Hartz.¹² The fact that in these cases of microfilarial granuloma there existed a pronounced or even severe eosinophilia points to the rôle of some kind of hypersensitivity in provoking these reactions. Such differences in the reactivity of the host are also found in other helminthic infections.⁶

SUMMARY

Microscopic examination of tissue removed from one of the enormously enlarged breasts of a 29 year old woman showed besides beginning adenosis the presence of edema, fibrosis, conspicuous dilatation of the lymph vessels and granulomatous endolymphangitis. In the connective tissue there were several small granulomas, each containing microfilariae but showing no tissue eosinophilia. Examination of the blood showed the presence of microfilariae. A diagnosis of elephantiasis of the breast, perhaps superimposed on some form of mammary hypertrophy was made. No other instance of microfilarial granulomas of the breast was found in the literature.

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LEIOMYOSARCOMA OF THE AMPULLA OF VATER ASSOCIATED WITH A TUMOR OF THE EIGHTH NERVE AND NEUROGENIC PERFORATION OF THE DUODENUM.

REPORT OF A CASE AND REVIEW OF THE LITERATURE*

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Leiomyosarcoma of the ampulla of Vater has heretofore been unreported. The infrequent occurrence of mesoblastic tumors of the duodenum is evident from a review of the literature. Shepherd¹⁵ and Duval,⁴ in 1908, presented a case of melanosaarcoma of the common bile duct and the ampulla of Vater. Moll,¹¹ in 1925, found a round cell sarcoma obstructing the duodenal biliary orifice causing acute pancreatitis. Of 66 cases of neurogenic sarcoma collected from the literature by Stewart and Copeland,¹⁶ to which 64 cases from the Memorial Hospital of New York City were added, none was of the gastrointestinal tract. Mumey¹² and Raiford¹³ reviewed sarcomas of the small intestine and attested to their rarity. Foshee and McBride,⁵ in 1939, added 1 case of leiomyosarcoma of the duodenum to the 5 cases assembled from the previous literature. The extreme rarity of this tumor and its association with an eighth nerve tumor has prompted the recording of this case. Of added interest in this case was its termination with perforation of the duodenum.

Leiomyomas of the small intestine are not uncommon and may be either single or multiple. The intestinal leiomyomas are usually asymptomatic and are found at postmortem examination by chance; features which indicate their preponderantly benign behavior.

A review of the literature yielded 6 cases of leiomyosarcoma of the duodenum varying in size from that of a "small tumor" to one the size of a "baby's head". In each of these cases the patient came to operation or postmortem examination because of the functional derangement directly caused by the tumor of the duodenum, whereas the tumor forming the basis for this report was an incidental finding.

The development of intestinal perforations with tumors of the eighth nerve has been infrequent. Cushing's¹ monograph on *Tumors of the Nervus Acusticus* describes no accompanying gastro-intestinal perforations. Horrax and Poppen⁷ reported five deaths in 35 cases of acoustic neuroma and Dandy³ noted 5 deaths in 46 cases without gastro-intestinal perforation or erosion.

Neurogenic perforation secondary to intracranial tumors as discussed by Cushing² and Masten and Bunts¹⁰ has usually been associated with tumors of

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the cerebellum rather than with those of the cerebellopontine angle. Sheehan,¹⁴ in reviewing the experimental work of Watts and Fulton,¹⁷ Hoff and Sheehan⁶ and Keller^{8, 9} concluded that hypothalamic lesions, particularly in the tuberal region, caused from 27.6 to 41.2 per cent of gastro-intestinal lesions while cerebral lesions exclusive of the hypothalamus caused from 2 to 4 per cent.

The background of neurogenic ulcerations and perforations of the upper gastro-intestinal tract has no specific pathologic entity, but has consisted of a variety of lesions which fall into two general groups: expanding lesions of the posterior fossa with proximity to the fiber tracts of the brain stem, and generalized intracerebral disease as in hypertensive encephalopathy, encephalitis or cerebral edema resulting in damage to the diencephalon.

There is no known relationship between leiomyosarcoma of the gastro-intestinal tract and tumors of the eighth nerve.

REPORT OF CASE

Clinical Data

Present illness. A white woman, 64 years of age, entered the Wisconsin General Hospital, on February 4, 1946, with a chief complaint of gagging and retching which occurred in the morning and which was unrelated to intake of food. This had appeared in the six months prior to admission and was accompanied by numbness about the left angle of the mouth, ataxia and diplopia. In the previous four years she had had tinnitus and deafness on the left side.

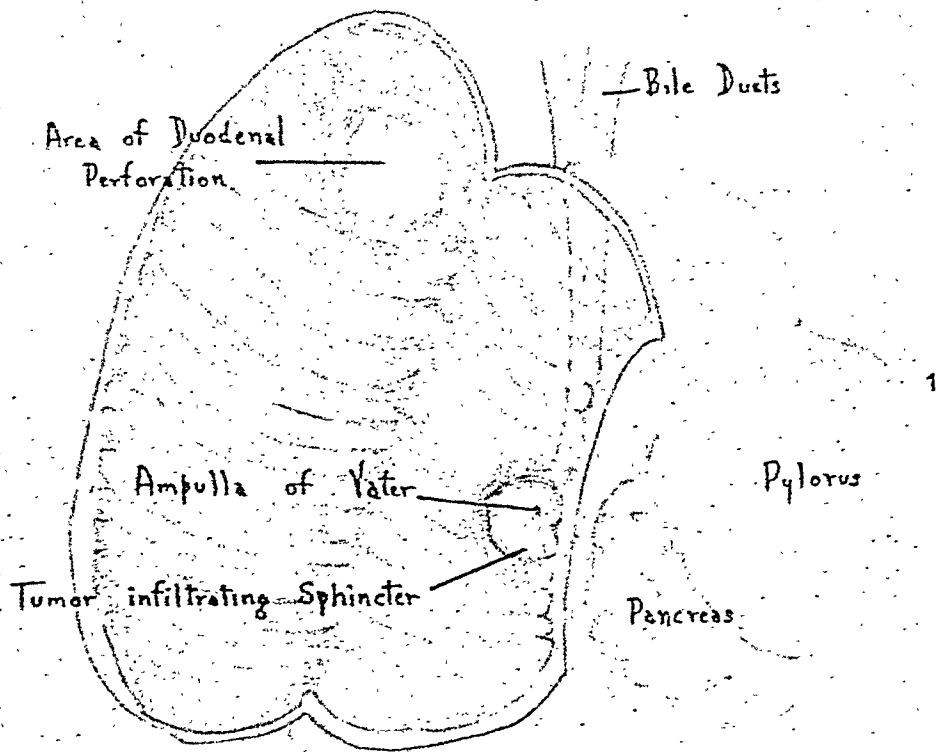
Physical examination. The patient was poorly nourished. Funduscopic examination indicated only arteriosclerotic changes. There was slight cardiac enlargement to percussion and the blood pressure was 166/105. An apical systolic murmur was present in the recumbent position. No abnormal abdominal mass was palpated, nor was tenderness elicited. Neurologic examination revealed the following: loss of left corneal reflex, partial atrophy of the left temporal and masseter muscles, slight weakness of the left orbicularis oculi muscle, shallowness of left nasolabial fold, absence of hearing on the left, nystagmus on gaze to the left, bilateral absence of gag reflex, asynergy on the left (finger to nose test), ataxia on placing left heel to right knee, bilateral pendular knee jerks; examination of the plantar reflexes was equivocal; the gait was ataxic with fall toward the left, and Romberg's sign was present.

Laboratory findings. The urinalysis, blood count, blood sugar, nonprotein nitrogen, prothrombin time, icterus index and van den Bergh reaction were all within normal limits. Test of gastric acidity revealed no free acid and 37 degrees of total acid. Blood Wassermann reaction was negative. Roentgen examination of the upper gastro-intestinal tract gave no evidence of any lesion and views of the cervical spine revealed considerable osteoarthritis. X-ray films of the skull showed definite enlargement of the left internal auditory meatus. Electrocardiogram was within normal limits. Examination of the cerebrospinal fluid demonstrated normal pressure, 450 mg. per cent protein, negative Wassermann reaction, positive Ross-Jones and Noguchi determinations and gold sol curve 0012332100.

Course. On February 11, a left suboccipital craniectomy was performed with an intracapsular removal of an acoustic neuroma. The following day the patient had regained consciousness and there was a left peripheral facial palsy, but water was swallowed without difficulty. On the fifth postoperative day, the patient responded less actively and lumbar puncture showed an initial pressure of 260 mm. of water. The cutaneous stitches were

FIG. 1. Drawing of the duodenal ulcer with perforation and tumor of the ampulla of Vater.

FIG. 2. Photomicrograph of the leiomyosarcoma of the ampulla of Vater. $\times 60$.



removed on the ninth day following surgery and at that time the initial pressure on lumbar puncture was 125 mm. of water. Later that same day a tarry stool was passed, but there was no vomiting, abdominal discomfort or abnormal abdominal physical findings. In the last four days of life, there was complete lack of responsiveness, generalized circulatory collapse partly checked by administration of blood transfusions and parenteral fluids and occasional apnea but no evidence of gastro-intestinal hemorrhage, peritonitis, or increased intracranial pressure. Death occurred on February 24.

Pathologic report. The sections of the surgical specimen showed a well vascularized tissue of different densities composed of interlacing slightly whorled bundles of long, narrow, spindle-shaped cells with some palisading of nuclei typical of an acoustic neurinoma.

Macroscopic Postmortem Findings

Autopsy was performed ten hours after death. The operative wound in the left suboccipital region and the underlying dura was well healed. The brain weighed 1320 gm. and was without evidence of flattening of the cerebral gyri. Tentorial and cerebellar pressure cones were absent. In the left cerebellopontine angle was a dark red homogeneous encapsulated mass 3 cm. in diameter which, on gross section, appeared to be composed largely of clotted blood; the peripheral portion was lighter in color and gave the impression of a capsule. Sagittal sections of the brain stem and medulla revealed no hemorrhages or areas of softening. A distortion of the left side of the floor of the fourth ventricle conformed to the overlying hemorrhagic mass. There was no evidence of compression of the aqueduct or ventricular dilatation. When the slightly distended abdominal cavity was opened, the granular and engorged peritoneum was found bathed with about 1000 cc. of dark yellow-brown, foul-smelling liquid; the omentum was firmly matted in the upper abdomen. On the anterior aspect of the first portion of the duodenum a round ragged perforation 2 cm. in diameter with necrotic margins was found. The duodenal contents had eroded through the overlying adherent omentum and had escaped into the peritoneal cavity.

The mucosa of the ampulla of Vater was elevated in its upper part by an eccentrically placed globular mass 1.5 cm. in diameter projecting 1 cm. into the lumen of the duodenum. It was very soft in consistency and the overlying adherent mucosa bled easily on light touch. The cut surface of the mass was meaty and red. The bile ducts were patent. No other tumors were found and the remainder of the autopsy gave no relevant findings.

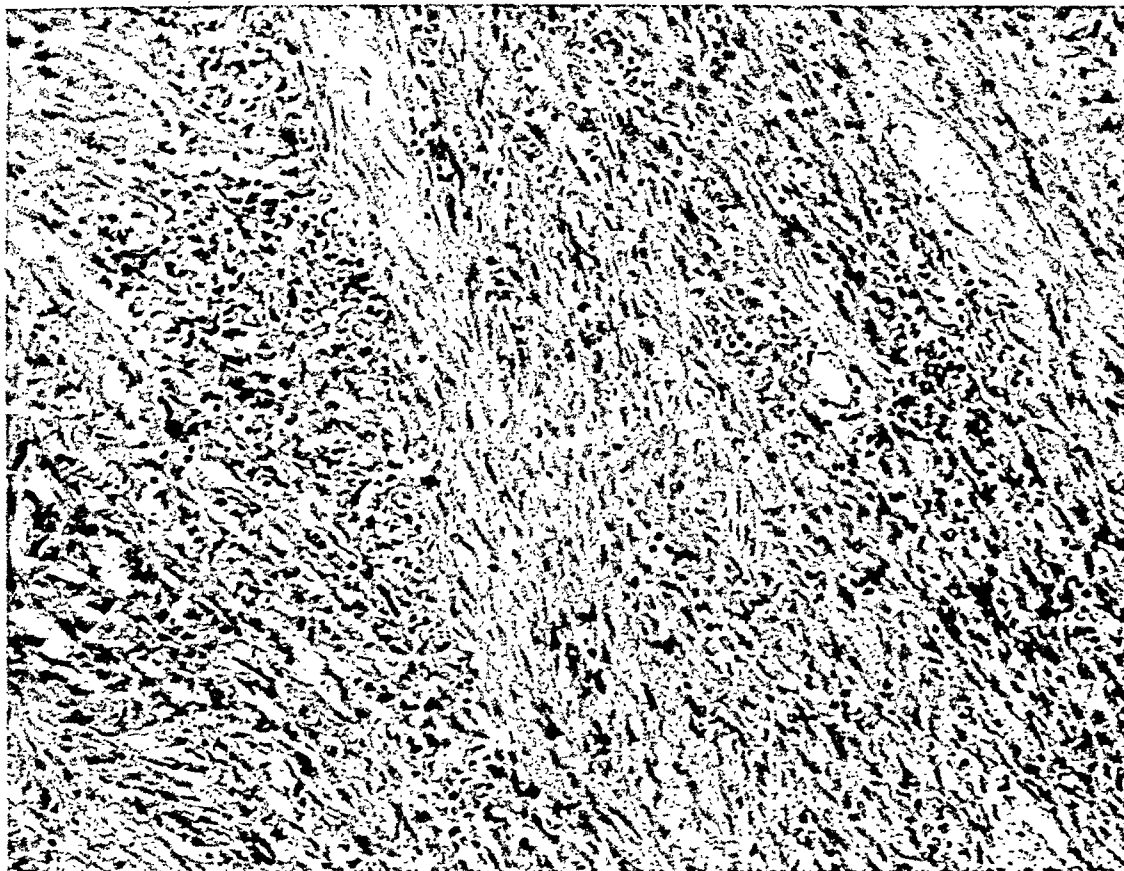
Microscopic Findings

The dark red homogeneous mass in the left cerebellopontine angle consisted of a peripheral ring of fibroblastic cells with considerable intercellular collagen; the elongated cells streamed in occasional whorl formation and there was a tendency toward nuclear palisading. At one point, the capsular attachment to the leptomeninges was identified but there was no meningeal invasion. The center of the tumor mass was made up largely of blood.

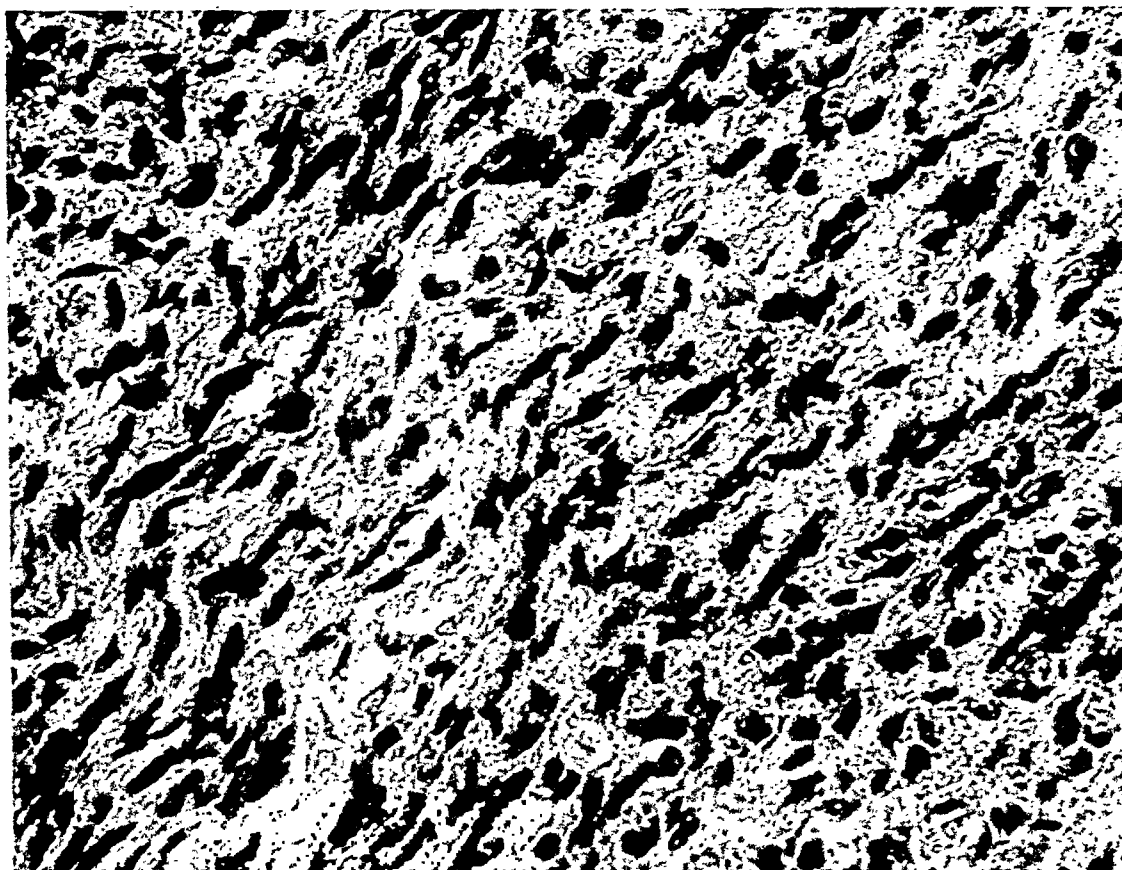
The edge of the perforated duodenal ulcer showed a fairly extensive fibrinopurulent exudate containing many polymorphonuclear leukocytes on the serosal surface, with some early fibroblastic reaction in the subserosa. The mucosal surface contained some lymphocytes and was locally ulcerated, necrotic and bile-stained. On the internal surface of the duodenum the margin of the ulcer began rather abruptly; the base had a superficial layer of fibrinopurulent exudate, a middle layer of hyaline necrosis and a deeper layer of granulation tissue. Many small vessels in the area contained thrombi.

FIG. 3. Photomicrograph of neoplasm. $\times 160$.

FIG. 4. Photomicrograph of neoplasm. $\times 550$.



3



4

Sections of the mass at the ampulla of Vater were stained with hematoxylin and eosin and with Mallory's phosphotungstic acid. The mass was situated between the muscle layers of the duodenum and consisted of spindle-shaped cells arranged in irregular bundles with occasional whorl formation (Fig. 2). The nuclei were very irregular in size, shape and staining reaction (Figs. 3 and 4) and occasionally suggested palisades. No mitotic figures were identified. Irregularly shaped nonstaining refractile bodies and groups of lymphocytes were scattered throughout the tumor tissue. The small vessels in several areas were occluded with thrombi of various ages. A poorly formed capsule was present with neoplastic cells invading the surrounding tissue in many places.

COMMENT

The majority of tumors of the ampulla of Vater are adenocarcinomas arising from the duct mucosa. In view of the abundant muscular layers of the duodenal wall it is surprising that tumors originating from muscle have not been more frequent. In this case, the tumor was presumably asymptomatic, but with the beginning sarcomatous changes it became a potential hazard to the patency of the biliary system as in the case of Moll.¹¹

Because of the local infiltration and the irregularity in size, shape and staining quality of the cells we have placed it in the malignant group with some reservation. The characteristic architecture of leiomyoma in some parts of the tumor would seem to indicate that it was originally a benign tumor. This, of course, cannot be proved. A diagnosis of neurinoma was considered, principally because of the associated acoustic neurinoma but was ruled out. Representative slides of the ampullary mass were studied by four well known pathologists, all of whom concurred in the diagnosis of leiomyosarcoma.

The occurrence of the perforated duodenal ulcer has given rise to interesting speculation concerning its pathogenesis. The histologic picture was characterized by lymphocytic infiltration of the mucosal surface and the ulcer margin revealed three layers: a superficial fibrinopurulent exudate, hyaline necrosis and a deeper layer of granulation tissue. The negative barium contrast study of the duodenum and the noncontributory history suggested the ulcer to be of recent origin. The well known difficulty of demonstrating anterior ulcerations of the duodenum by x-ray, however, prevented an accurate estimation of the age of the process. The association of the acoustic neuroma in this case may have been coincidental but it has been noted clinically that tumors of the posterior fossa have been more frequently associated with neurogenic gastro-intestinal perforations than expanding lesions elsewhere. As the eighth nerve tumor did not cause internal hydrocephalus and secondary diencephalic pressure and damage, the effect may have been produced by stimulation of the fiber tracts in the brain stem or the adjacent vagus nerve either by direct pressure or more likely by edema of the brain stem which stimulated the vagal nuclei.

SUMMARY

A case is presented of leiomyosarcoma of the ampulla of Vater associated with a tumor of the eighth nerve and neurogenic perforation of the duodenum.

Acknowledgments. The authors wish to thank Dr. T. C. Erickson for his kind permission to report this case and Dr. D. M. Angevine and Dr. Gordon Ritchie for aid in the preparation of the manuscript.

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EDITORIALS

OBTAINING PERMISSION FOR AUTOPSY

In spite of the generally recognized value of autopsies in the advancement of medical science, pathologists often find resistance on the part of families and funeral directors in obtaining consent for autopsy. Sometimes refusal springs from misunderstanding or misinformation, sometimes from previous embarrassing or unpleasant experiences of the family. Wherever a foundation of mutual understanding and reasonable cooperation has been established between pathologists and funeral directors, the situation in respect to gaining consent for autopsies has been considerably improved.

The pathologist has certain interests and requirements that he desires to fulfill. To secure the utmost scientific benefit from each death, he wishes to ascertain all facts concerned with that death. This is an obligation to the family of the deceased, to future patients and to society. The data obtained at autopsy provide reliable recorded information concerning the causes of death and the nature of the various disease processes; confirm or amend the opinions formed by physicians during the life of the patient, so that they may serve other patients with greater confidence and skill; reveal to physicians continually the physical changes in the interior of the body which are associated with disordered behavior during life; advance human knowledge concerning the nature of disease in general and exercise a constant influence to improve hospital services and to correct existing deficiencies in medical care.

The funeral director has certain interests and requirements that he desires to fulfill. He must meet legal requirements. He must care for the body and render his services in a manner that will be entirely satisfactory and comforting to the family. He must be governed by traditional folk ways and religious customs. Practically every funeral director recognizes the value and importance of autopsies. Whatever objections he may have to them are usually founded upon those practices which prevent him from meeting his obligations or which cause him unnecessary hardship or embarrassment. Generally speaking, funeral directors who are most cooperative with pathologists are those who enjoy good standing in their profession.

The interval of time between death and performance of autopsy is important to the pathologist as postmortem changes within the body affect the gross and microscopic appearance of tissues. The longer the lapse of time between death and embalming, the more difficulties and handicaps the funeral director must overcome. Since the proper appearance of each body is important to the funeral director in the maintenance of his professional status, the time element should be given every consideration by the pathologist. Any delay in preparing the body for viewing by the family and friends only adds to the emotional strain and may place the funeral director in an unnecessarily embarrassing position. In addition, there is a schedule of operation which must be maintained in each funeral home. The funeral director makes arrangements with the family for

the funeral program according to advice from the pathologist or hospital as to when the body will be available. Any delay will force changes in this program, which are difficult to make and, which may cause the family to question its judgment in granting permission for autopsy.

Permission for an autopsy should be requested at the earliest possible moment. The family should be given a proper explanation of the reasons for the performance of an autopsy. The signed autopsy permit should state the extent of the autopsy and whether any organs are to be retained for study; these provisions protect both the pathologist and the funeral director. Under no circumstances should a family be coerced into signing a permit and it is improper to threaten to call in the coroner or medical examiner in order to secure a signature.

In respect to the actual autopsy operation there are certain requisites of the funeral director to which the pathologist should give considerate attention. Nurses and hospital attendants should be given instructions to keep the head and shoulders of the body elevated at all times to prevent postmortem staining of the face and neck and to refrain from tying a bandage or towel around the head in order to avoid marks or unnatural wrinkles in the skin.

It is obvious that incisions should be made which will not be visible to the family afterward. The mental anguish caused families when the incisions and sutures cannot be masked by the funeral director is responsible for some of the resistance to autopsies. When the brain is to be examined, an angular cut should be made in the calvarium to prevent it from slipping and also to assist in making the cut less obvious. Pathologists should also thoroughly understand that the funeral director depends upon the circulatory system for the distribution of preservative solutions. Therefore any undue disruption of that system, or the careless cutting of main arteries and veins, greatly complicates and handicaps the embalming operation. The severed branches of the arch of the aorta, the iliac arteries, as well as the vertebral and internal carotid arteries, should be as long as possible, tightly ligated and left attached, each to a long string. In any examination of the neck, care should be exercised to protect the external carotid arteries since they carry the preservative solutions to the face. When the spinal cord is to be examined, it should be approached from the anterior aspect. This will eliminate the necessity of turning the body face down and thus will prevent disfigurement and hypostatic staining of the face. The pathologist who ignores these factors helps to build resistance to autopsies. The pathologist who takes the above factors into consideration will generally be the pathologist who practices a careful technic in the performance of his autopsies.

A completed death certificate must be filed for each death. This is the responsibility of the funeral director who, in return, secures a burial or transit permit which is essential to his program of procedure. Hospital staffs would render valuable assistance if all information required on the death certificate were secured at the time the patient entered the hospital. This information plus that required of the attending physician should be promptly available for the funeral director when he calls at the hospital for the body. The funeral director, or his agent, should present to the hospital acceptable evidence that

he has been authorized by the family to take charge of the body. Hospital employees should not give information to unauthorized persons in regard to patients critically ill or those who have died in the hospital. Employees guilty of infractions of this rule should be subject to discipline.

Any reasonable analysis of the problems connected with obtaining permission for autopsy will indicate that, with mutual understanding between funeral directors and pathologists, progress can be made in the right direction. This understanding and the resultant cooperation can be best promoted and developed at the local level through conferences between leaders of both groups.

Chicago

S. A. LEVINSON*

MAYNARD C. WELLER*

SHORTAGE OF REGISTERED MEDICAL TECHNOLOGISTS

At no time has the shortage of qualified medical laboratory workers been felt as acutely as at present. Many factors have operated to produce this shortage; chief of these is probably the decrease in training of medical technologists during the war. It is to the credit of the pathologists of the United States and Canada that they undertook the training of those workers who were available, but there appears to have been a deficit of approximately fifty per cent in the minimum number of trainees needed for positions as Registered Medical Technologists.

The shortage of adequately trained technologists has had far-reaching effects, one of which has been the rapid and marked increase in the salary of medical technologists and, another, the widespread employment of nonregistered laboratory workers. Partly as a consequence of the ready employability of almost any type of laboratory worker, and partly because of the availability of funds through the G. I. Bill of Rights, commercial schools have had a great stimulus and are turning out large numbers of "graduates" with grossly incomplete "training" and with practically no actual technical experience. The full effect of the encroachment of these incompetent workers in the field of medical technology has not yet been felt. It is imperative that the training of medical laboratory technicians by acceptable standards must be increased to the level at which at least a major part of the demand for Registered Medical Technologists will be satisfied. This is a responsibility which must be met by the approved schools of medical technology.

The chief obstacle to the training of adequate numbers of student technologists is that in many parts of the country there are not enough applicants to fill the classes. On the other hand, some approved schools report a plethora of applicants. In an effort to establish a more equitable distribution of these prospective students, the Registry of Medical Technologists has undertaken to act as a clearing house. All schools which have extra applicants are urged to refer

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them to the Registry immediately, so that the Registry may in turn direct them to schools which have vacancies. If all pathologists will cooperate in this endeavor, there should be a considerable increase in the enrollment of student technologists.

Furthermore, all other methods of increasing interest in medical technology as a vocation should be adopted. Both pathologists and representative Registered Medical Technologists should take every possible opportunity to speak to groups of high school students and urge them to consider this important field in choosing their college course. College students who have been interested in science and who have the necessary credits should be urged to apply now for training in approved schools. The Registry of Medical Technologists will be glad to send material to anyone interested in preparing talks to selected groups, or radio talks which can be used over local stations. No opportunity should be overlooked to do everything possible to satisfy the demand for qualified medical technologists at the very earliest time.

Muncie, Indiana

LALL G. MONTGOMERY

SELECTED ABSTRACTS

Present Status of the Problem of Amebiasis. EDWIN C. ALBRIGHT and EDGAR S. GORDON. Arch. Int. Med., 79: 253-271, 1947.

Basing their opinion on more than three years' experience with the armed forces in the Pacific theater, the authors of this excellent paper believe that, while most of the personnel infected with malaria and filariasis were brought under medical care, most of those affected with amebiasis were not. Thus the returning troops, added to the already considerable percentage of infected persons in this country, increased the gravity of an already serious problem.

The epidemiology and morbid anatomy of amebiasis are reviewed followed by a discussion of the carrier state. The authors prefer to use the term asymptomatic passer of cysts, since they believe that "carrier" is misleading, as it connotes absence of lesions, and they believe that whenever cysts are present in the stools, there is definite disease qualitatively identical with amebic dysentery, and that the difference is only quantitative.

Among the diagnostic procedures, proctoscopic examinations are stressed. If microscopic examinations of material removed in this way fail to reveal the organism, at least three naturally passed stools and one following a saline purgative should be examined before giving a negative report.

We are reminded of the fact that emetine acts only on the amebae in the tissues and that for complete eradication of the cysts from the bowel one must resort to one of the enteric drugs such as chiniofon, diodoquin, vioform, or carbarsone. A rigid schedule is outlined for the patient receiving emetine because of its toxic effect on heart and voluntary muscle.

The use of the sulfonamides and penicillin, as described by Hargreaves, is recommended as preparatory medication in the use of amebicidal drugs in order to combat the secondary bacterial invaders which often complicate enteric infections.

Rochester, New York

W. S. THOMAS

Dysentery. A Progress Report for the Years 1942 to 1946. A. J. WEIL. J. Immunol., 55: 363-405, 1947.

This is the second article of the series of reports on bacillary dysentery published by Weil. The paper contains a summary of 317 articles, critically reviewed.

First the nomenclature of *Shigella* is discussed, with special regard to recent publications by Stuart, Wheeler *et al.* and by Weil. The term "shigellosis" is much favored. Attention is called to the biochemical differentiation of *Shigella* strains but the occurrence of aberrant reactions is emphasized. The importance of serologic typing is brought out and the difficulties due to phase variation, labile antigens, intergrading forms and cross relations are discussed. The types of *Shigella* are fully listed, with many references to their geography. Weil feels that *Sh. alkalescens* is pathogenic, while the ability of *Sh. dispar* to produce disease has not been proved as yet. Slide agglutination is considered superior to other methods of *Shigella* typing. Much attention is paid to the *Shigella* toxin. The somatic antigen acts as an aggressin and inhibitor of opsonin. The Shiga exotoxin has an adrenalin-like effect. Immunization against shigellosis is difficult, because of the great variety of strains. The unreliability of agglutination tests using the serums of patients is discussed. The dangers in the sulfonamide treatment of shigellosis are emphasized. Dietary management and simple therapeutics, as with pectin, for example, is recommended whenever possible. Sulfadiazine is very effective. The author believes that it is not possible to make a definitive statement on the clinical efficacy of streptomycin in shigellosis at this moment.

The paper is an excellent, comprehensive review, presented with much constructive criticism.

Chicago

OSCAR FELSENFELD

Platelet Fragility in Man. LEWIS R. DAY. *Proc. Soc. Exper. Biol. and Med.*, 64: 109-111, 1947.

Investigations on the fragility of platelets in normal and hemophiliac males were undertaken, utilizing a method similar to the study of the hemolysis of red blood cells in varying hypotonic solutions of saline. The author demonstrated that solutions containing platelets exposed to hypotonic saline more rapidly clotted citrated blood when recalcified than did platelets in isotonic saline. He further showed that the more hypotonic the saline used, the more rapid was the coagulation. In addition, the longer the platelets were exposed to the hypotonic solution, the more rapid was the coagulation of the blood after recalcification. When these methods were applied to blood from a known hemophiliac male, the clotting time was shortened when the platelets from the patient were lysed with hypotonic solution. The author suggests that the abnormality in the blood clotting mechanism in hemophilia is due to some inherent change in the platelets, rather than to a deficiency in the plasma. He does not, however, discuss the mechanism of reduction to normal of coagulation time in hemophiliac patients who have received injections of fraction I of the plasma globulins.

Brooklyn, New York

LEO M. MEYER

Thiouracil in the Treatment of Leukemia. O. C. HANSEN-PRUSS. *Proc. Soc. Exper. Biol. and Med.*, 64: 496-500, 1947.

The author reports his observations in leukemic patients having the following clinical features of hyperthyroidism: increased metabolic rate, tachycardia and decreased blood cholesterol. He found an inverse ratio in the severity of the disease to blood cholesterol levels; that is, the values for the latter dropped rapidly as the number of myeloblasts increased. As remissions occurred following x-ray treatment, the serum cholesterol values rose above 150 mg. per cent. Since thiouracil had proved so efficacious in controlling simple cases of hyperthyroidism the author treated 6 patients with chronic myeloid leukemia and 1 with acute myeloblastic leukemia with varying doses of the drug for periods up to ten months. In summary, *there were no changes in the metabolic rate, blood picture or bone marrow.* In 5 of the patients the serum cholesterol was not raised, while in the remaining 2 there was only a slight transitory increase. In no case was the clinical course altered. Similar negative results in the treatment of 4 cases of chronic myeloid leukemia, 1 of acute myeloblastic leukemia and 1 of chronic lymphoid leukemia were reported by Limarzi, Kulasavage and Pirani (*Blood*, 1: 426-445, 1946).

LEO M. MEYER

The Use of Folic Acid in Sprue. R. M. SUAREZ, T. D. SPIES and R. M. SUAREZ, JR. *Ann. Int. Med.*, 26: 643-667, 1947.

The authors report their experiences with the use of folic acid in 50 patients with sprue. Twenty-two were in the acute phase of the disease, while the remaining 28 were in remission and were transferred from parenteral liver therapy to oral folic acid. The patients selected fulfilled all the criteria for the diagnosis of sprue and were critically examined for extraneous disorders. Five to 500 mg. of folic acid was given in order to determine the optimal therapeutic dose. Many of the patients were maintained on a basic sprue diet in order to test thoroughly the efficacy of the drug. The clinical response in all the acute cases was dramatic. Within three or four days the appetite increased, diarrhea and gaseous distension became less marked, the tongue was less sore and the patient voluntarily stated that he felt better. *Reticulocytosis always occurred* (in one patient it was 51.8 per cent), *and the hemoglobin and red blood cell values gradually increased.* The best clinical and hematologic results were obtained in patients who were changed from the "preliminary sprue diet" to a full diet, and in those who were started immediately on the full diet and given folic acid in doses of from 5 to 20 mg. per day. In the group of 28 patients previously treated parenterally with liver or orally with brewer's yeast in whom treatment was changed to oral administration of folic acid, 19 experienced some subjective and hematologic improvement, while the condition in the remaining 9 was unchanged. The authors conclude that the daily oral

administration of 10 mg. of folic acid in conjunction with an adequate diet high in protein and low in carbohydrate and fat will probably produce the best clinical and hematologic results in the treatment of sprue.

LEO M. MEYER

The Treatment of Lymphoblastic Leukemia with Crude Myelokentric Acid. F. R. MILLER, P. A. HERBUT and H. W. JONES. *Blood*, 2: 15-39, 1947.

The authors describe the use of extracts made from urine or feces of patients with chronic myeloid leukemia in the treatment of 8 children suffering from acute lymphoblastic leukemia. There were four controls. The treated subjects showed various degrees of clinical improvement, reduction in the size of lymph nodes, liver and spleen, fall in the total leukocyte count and a concomitant drop in the number of lymphoblasts. Transfusions were done less frequently and the bone marrow showed evidence of myeloid stimulation. Of the 8 treated patients, 7 died, mainly of intercurrent infections. In many instances, there was an insufficient amount of extract available to make continuous treatment possible. In addition, the authors suggest that increasing doses may be necessary since the individual patients may build up a tolerance for the extract. Autopsy findings disclosed significant changes in the lymph nodes, liver, spleen and bone marrow. These consisted mainly of a washing out of the infiltrative process in these organs with decreased cellularity and increase of reticulum and fibrous tissue. The lymphocytes remaining were distorted and pyknotic. This reviewer has had the opportunity to study these slides and is most favorably impressed with the changes described.

LEO M. MEYER

Changes in State of Hemoglobin in Erythrocytes During Hemolysis. D. L. RUBINSHTEYN and Z. P. BARINOVA. *Biokhimiya*, 10: 243-257, 1945.

The authors cast some doubt on the currently prevailing concept that changes in the permeability of the erythrocytic membrane form the fundamental basis for hemolysis. According to their studies, *the basic mechanism of hemolysis consists in the breakdown of a labile compound of hemoglobin and stroma*, for which they propose the name "hemostromatin". This compound is probably of colloidal nature, but further studies are required to determine its exact composition. Its breakdown constitutes an irreversible process. The degree of hypotonicity plays an important rôle in the breakdown of hemostromatin, the greater the hypotonicity, the more pronounced the decrease of the relative hemoglobin content of hemostromatin; conversely, if the stroma is not markedly damaged, the smaller the hemoglobin content of erythrocytes, the higher the erythrocytic resistance to hypotonic solutions. This finding may clear up the apparently unexplained fact that in hypochromic anemias the erythrocytes usually show a distinctly increased osmotic resistance. But even with maximal hypotonic solutions used (0.05 per cent sodium chloride), the stroma retained up to 15 or 20 per cent of the initial hemoglobin content. Hemoglobinolysis occurs as the result of partial breakdown of hemostromatin; if this breakdown reaches a certain critical level, hemoglobinolysis is followed by stromatolysis. These phenomena represent two stages of the same process. Thus, hemolysis results from breakdown of hemostromatin as well as from changes in the permeability of the membrane which permit the escape of liberated hemoglobin.

This study should be confirmed by other investigations. The detection of properties of hemostromatin may furnish a key to a better understanding of the physicochemical structure of erythrocytes.

Terre Haute, Indiana

LEON L. BLUM

Funktionelle Knochenmarkspathologie (normale und pathologische Wechselbeziehungen zwischen Markparenchym, Nerven-, Gefäß- und Knochensystem). KARL ROHR. Schweiz. med. Wchnschr. 75: 773-777, 1945.

In the interrelationship between bone marrow and neuro-endocrine system, opposite effects of stimulation of the sympathetic and parasympathetic systems are stressed. Stim-

ulation of the sympathetic division produces a blood leukocytosis, acceleration of cell maturation, decreased volume of marrow and increased fat volume.

Only the mature leukocytes get into the circulation due to their ameboid motion. This explains the discrepancy between hyperplasia of the bone marrow and cellular depletion of the peripheral blood. The presence of immature cells in the circulating blood requires the assumption of extramedullary hematopoiesis, especially in the spleen. In the spleen, close communications between the pulp and the blood vessels exist and immature cells can easily get into the circulation. Rohr stresses the fundamental difference between aleukemic and leukemic myelosis. According to the author, the leukemic picture always indicates extramedullary hematopoiesis, whereas the aleukemic stage indicates that the pathologic process is still limited to the bone marrow. In infections, toxic states and bone marrow metastases, the immature cells come, almost without exception, from foci of extramedullary hematopoiesis (spleen) and are not "irritation" forms. The author considers the "leukoses" not as reactive hyperplasias, but as neoplastic processes. Leukemic cells are, therefore, not normal, but neoplastic. Attention is called to the necessity of differentiation between two kinds of giant cells in the bone marrow: (1) a true bone marrow giant cell, the megakaryocyte, and (2) a bone giant cell, the polykaryocyte (osteoblast, osteoclast). A chart illustrates the pathogenesis of giant cells within the reticulo-endothelial system.

This is an interesting review and the interpretation of the significance of immature cells in the peripheral blood merits consideration.

LEON L. BLUM

Studies on the Nature of Antibodies Produced in vitro from Bacteria with Hydrogen Peroxide and Heat. E. C. ROSENOW. J. Immunol., 55: 219-232, 1947.

Production of agglutinins and precipitins by prolonged heating of gram-positive bacteria in sodium chloride solution-suspensions, has been reported. Much less heat was required if an oxidizing agent such as hydrogen peroxide was added to both gram-positive and gram-negative organisms, and agglutinins and precipitins were produced in high titer. These antibodies were specific but reacted more with closely related organisms and their polysaccharides than did the natural antibodies. They were not bactericidal *in vitro* but were definitely protective *in vivo*. They were stable in acid solution but their titer dropped rapidly when protein was added. They had great avidity for particulate matter and were not filtrable. They were dialyzable through cellophane and distilled over on boiling.

Agglutinins could also be produced from globulin with and without bacteria by hydrogen peroxide and heat. High pressure at ordinary temperature had no effect. Dilution with sodium chloride solution reduced the titer proportionately, but dilution with globulin, albumin or serum caused a much greater reduction in titer, while heating or conjugation with globulin, serum or albumin caused the titer to increase markedly.

It is suggested that the destruction of antigen *in vivo* by reticulo-endothelial cells may be due to enzymatic oxidation of antigen into diffusible antibody and that the diffusible antibody combines with normal globulin *in vivo* in lymphocytes, reticulo-endothelial cells and other cells and in serum to form antibody-globulin. Experimentally, minute amounts of bacterial antigen sufficed for production of diffusible antibody and extremely small amounts of diffusible antibody may suffice to cause normal globulin to take on the properties of antibody-globulin. This suggests that possibly most of the weight of natural antibody-globulin represents globulin and only a small fraction is antibody from antecedent antigen.

Dallas, Texas

J. H. BLACK

Primary Carcinoma of the Liver. ROBERT M. HOYNE and JAMES W. KERNOHAN. Arch. Int. Med., 79: 532-554, 1947.

The authors present 31 original cases and survey the literature for pertinent clinical and pathologic features followed by analysis of their cases.

In 16,303 necropsies at the Mayo Clinic, the incidence of this tumor was 0.19 per cent.

Twenty were of the parenchymal cell variety; eleven were of the cholangiomatous type. Their series confirmed the fact that this is a tumor of an older age group, the average age for men being 58.5 years and for women 48.9 years. They are in agreement with the statement that chronic irritation of the liver is a prominent causative feature. Of the authors' cases, 75 per cent of the hepatomas were associated with cirrhosis, but in only 18.2 per cent of the cholangiomatous type was cirrhosis present. In one case there was hemochromatosis. The clinical features included weakness and abdominal pain and distention as the outstanding symptoms, and progressive enlargement of the liver as the outstanding physical sign, although the latter was present in only one-half of the cases. The gross appearance of the tumor cannot be used to distinguish between the two main varieties. Microscopically, however, there are four differential features: arrangement of cells, cell type, type of stroma and secretion of bile. Cholangiomas tended to metastasize more widely and earlier than hepatomas in their experience. Lymph nodes and lungs were the most frequent sites of metastasis. Most of the patients died within a few months after the onset of symptoms. The only treatment is resection of the primary neoplasm before metastasis has occurred.

The authors do not mention hemoperitoneum as a prominent secondary feature of the disease. The reviewer has been impressed with the presence of this complication and its importance as a clinical diagnostic sign.

Detroit

BERT E. STOFER

Changes in Kidneys in Carbon Tetrachloride Poisoning, and Their Resemblance to Those in "Crush Syndrome". W. W. Woods. J. Path. and Bact., 58: 767-773, 1946.

Woods reports 3 fatal cases of carbon tetrachloride poisoning in confined ship spaces following dry-cleaning of clothes with Pyrene from fire-extinguishers. After a group of men had cleaned clothes once a week for three months, three of the men fell ill and one died. In two other fatal cases, the patients showed signs of profound poisoning four and three days, respectively, after a single clothes-cleaning session. In all cases the uremic state preceded death.

Glomerular alteration was largely of capsular epithelium, with albuminous debris in the spaces. Tubules, with the exception of the proximal convoluted tubules, suffered most, with changes indistinguishable from those described in the crush syndrome. These included degeneration, loss of cells, and early regeneration in the ascending parts of Henle's loops and distal convoluted tubules; there were also casts from fragmented erythrocytes (?), and café-au-lait pigmented casts. Two cases had tubulo-venous communications (Dunn *et al.*, Lancet, 2: 549-552, 1941). It is suggested that the shunting of blood from cortex to medulla causes pressure changes with rupture of veins into tubules. Such communications would be responsible for the clinical and histologic features of both carbon tetrachloride poisoning and the "crush syndrome".

Fort Wayne, Indiana

S. M. RABSON

Heterologous Transplantation of Uro-epithelial Tumors. Part II. Transplantation of Bladder Tumors. M. S. HOVENANIAN and C. L. DEMING. Yale J. Biol. and Med., 19: 149-153, 1946.

The authors transplanted two neoplasms of the human urinary bladder into the anterior eye chambers of guinea pigs. One tumor was a papilloma which had recurred after a period of two and one-half years; biopsies failed to show cytologic irregularities or stromal invasion. The second tumor was a papillary carcinoma with infiltration of the stroma.

Both heterologous transplants developed into solid masses of transitional epithelium, closely resembling each other and the parent tumors. The authors conclude that the malignant nature of the simple bladder papilloma has thus been demonstrated, and they recommend the use of this biologic test for establishing the nature of such neoplasms.

This report is based on the studies of H. S. N. Greene who introduced the method of heterotransplantation as valid criterion of malignant growth. Further contributions may be expected from this approach in instances where morphology alone fails to give a conclusive answer.

Chicago

KURT STERN

Carcinogenic Substances in Human Tissue. I. HIEGER. *Cancer Res.*, 6: 657-667, 1946.

Lipoid extracts prepared from lung, kidney, muscle and liver of cancerous and noncancerous human subjects were fractionated and tested as to their carcinogenic potency on mice. Subcutaneous injections initiated development of sarcomas in 8 mice after fifteen to twenty months. With one exception, carcinogenic potency was exhibited only by the unsaponifiable cholesterol-rich fraction. Organs from cancerous as well as from noncancerous individuals yielded potent extracts.

Correlating previous similar work with his own observations, the author gave consideration to the possibility that these carcinogens might be artefacts, *i.e.*, might be formed during the processing of the extracts. Experiments of the author testing this possibility, so far, have shown negative results.

Further investigations, particularly as to the chemical nature of the potent agent, are being undertaken by Hieger and his collaborators. Chemical characterization of carcinogens from human tissue will doubtless represent an important step forward toward the understanding of cancer development.

KURT STERN

BOOK REVIEWS

Recent Advances in Clinical Pathology. Produced under the auspices of The European Association of Clinical Pathologists. General Editor, S. C. DYKE, D.M. (Oxon), F.R.C.P. (Lond.); Section Editors: Bacteriology: R. CRUICKSHANK, M.D. (Aberd.) F.R.C.P. (Lond.), Biochemistry: E. N. ALLOTT, B.Sc., B.M., B.Ch. (Oxon), F.R.C.P. (Lond.), Haematology and Cytology: B. L. DELLA VIDA, M.D. (Rome), Histology: A.H.T. ROBB-SMITH, M. D. (Lond.). 468 pp., 34 plates, 19 figs. \$5.50. Philadelphia: The Blakiston Company, 1947.

Many clinical pathologists who prior to the last war were driven from various European countries by political developments found refuge in Great Britain where they rendered valuable service. Together with their British colleagues, they founded the European Association of Clinical Pathologists. At the meeting of the Association in July, 1944, the idea took shape to publish in book form a symposium on recent advances in the practice of clinical pathology in Great Britain. The volume was edited by S. C. Dyke, the president of the Association. Four contributions are from Czechoslovakia, one each from Italy, Australia and South-Africa, thirty-four from England. The book is divided into four sections.

Bacteriology, edited by Cruickshank, contains chapters on laboratory diagnosis of enteric infections, typhus fever, sore throat, primary atypical pneumonia, pertussis, tuberculosis, brucellosis, anaerobic, staphylococci, leptospiral and venereal infections, classification of streptococci and laboratory control of chemotherapy.

Biochemistry, edited by Allott, includes liver function tests, prothrombin time, gastrointestinal diseases, Addison's disease, nutritional deficiencies, sulphonamides, photo-electric colorimeters and micromethods of blood analysis.

Hematology and cytology, edited by Della Vida, covers hematologic nomenclature, the myelogram, sedimentation rate, pernicious anemia, semen, sputum and pleural fluid.

Histology was edited by Robb-Smith and embraces discussion on recent developments in aspiration biopsy, cell counts in serial biopsies of carcinoma, biopsy of lymph nodes, testicles, endometrium, skin, peripheral nerves, wet film technic in neurosurgery and clinical and postmortem pathology of encephalitis. In the concluding chapter modern histologic techniques are reviewed.

Two aspects of each subject have been emphasized successfully throughout the book: the recent and the practical. As a result the clinical pathologist in this country will find a condensed presentation of advances in his field. The large number of contributors has resulted unavoidably in unevenness, but they are, on the whole, not too conspicuous.

It is gratifying to see that the section on histology occupies more space (140 pages) than any of the others. Robb-Smith's delightfully written foreword expresses so well recent trends in clinical histopathology that quoting him verbatim may not be out of place. He speaks of "the renaissance of morbid histology—the integration between the histologist and the clinician in the illumination of obscure corners, whether of diagnosis or prognosis, of disease". Robb-Smith's chapter on the lymph node biopsy presents his own interesting classification of lymphatic lesions employing successfully the inductive method by analyzing the histologic findings step by step for the purpose of making a diagnosis, as contrasted with the customary way of making the diagnosis first and following it up with a description.

Lack of space does not permit mention of further details of the many stimulating and informative chapters in this remarkable book. Every clinical pathologist will find in it a reliable source of valuable information.

Chicago

I. DAVIDSOHN

Color Atlas of Hematology. By ROY R. KRACKE, M.D., Dean and Professor of Clinical Medicine, Medical College of Alabama, Birmingham, Alabama. 204 pp., 32 full color plates, 3 black and white plates. \$5.00. Philadelphia: J. B. Lippincott Company, 1947.

The author has taken much material and most of the color plates for this volume from his larger work, *Diseases of the Blood*. Besides 32 color plates illustrating the morphology of blood cells and various hematologic conditions, the manual contains a brief clinical description and treatment of various diseases. There is a short chapter on the definition of hematologic terms. In the chapter on the origin and development of blood cells, the author follows the polyphyletic theory of hemopoiesis which is illustrated by a color plate. Several chapters are devoted to the morphology of blood cells in normal and pathologic states. The clinical description of hematologic conditions includes chapters on the anemias, leukemias, hemorrhagic diseases, leukopenic syndromes and miscellaneous diseases of the blood and blood forming organs. There is a section on conditions presenting splenomegaly and the indications for splenectomy. Although the author states that megaloblasts are present in small numbers in normal bone marrow, these cells are listed (page 125) as having zero percentage in the table on the differential count of normal bone marrow. This is probably a typographical error.

There are chapters on blood parasites, including malarial parasites, blood findings and pictures in various laboratory animals, and good descriptions of the more common hematologic technics. On page 158 in the section on hematologic technic, the examples cited for the calculation of mean corpuscular volume and mean corpuscular hemoglobin are misleading due to typographical errors in the numbers used for determining these corpuscular standards. The last chapter is a summary of the hematologic findings in various diseases and conditions and it is followed by several pages of bibliography.

Hematologists with a keen morphologic perspective will question many of the color plates on the basis that they do not illustrate the delicate cytologic differences necessary for the proper identification of blood cells. Although hematologists will necessarily consult more comprehensive works on the subject, the manual contains much information in a concise form on many blood dyscrasias which should prove valuable for medical students, laboratory technicians and general practitioners of medicine.

Chicago

LOUIS R. LIMARZI

Surgical Pathology. Ed. 6. By WILLIAM BOYD, M.D., Professor of Pathology, The University of Toronto. 858 pp., 530 figs., 22 in color. \$10.00. Philadelphia and London: W. B. Saunders Company, 1947.

The sixth edition of Boyd's *Surgical Pathology* represents a revision of the previous edition designed to bring the reader abreast of the advances of the past five years in this field. New material includes surgical pathology of the heart, tumors of the larynx, pinealoma, Bittner's milk factor in relation to breast carcinoma, avitaminosis in cancer of the mouth, the Papanicolaou vaginal smear method in diagnosing carcinoma of the cervix, fibrous dysplasia of bone, inflammatory nodules of muscle in chronic arthritis and fibrositis of the back.

The value of this attractively published text is enhanced by 530 exceptionally clear and instructive illustrations including 22 in color. Additional well selected references for study have been added to the bibliographies at the end of each chapter, which are arranged according to subject matter.

The reader not already acquainted with this popular book will find a pleasing literary style unique in didactic medical literature. This, together with a practical concise presentation of the subject with close correlation of the important medical and surgical aspects of the diagnosis and treatment of disease, make it one of the most readable treatises on the subject and also a valuable asset in the library of the student, surgeon, or general pathologist.

Detroit

K. T. MILLER

The Acute Infectious Fevers. An Introduction for Students and Practitioners. By ALEXANDER JOE, M.D., D.P.H., Medical Superintendent, City Hospital, Edinburgh; Lecturer on Infectious Diseases, University of Edinburgh. 276 pp., 64 figs. \$4.50. Philadelphia: The Blakiston Company, 1947.

Fifteen topics have been chosen for inclusion in this little volume and are presented in a manner styled for appeal chiefly to, "students, junior medical officers in hospitals and practitioners. . .". The approach is primarily clinical and reflects the author's unrivaled experience in the bedside care of patients with common infectious states. Outstanding are the chapters dealing with streptococcic disease and diphtheria, although all are excellent and are phrased in a delightfully informal manner which makes reading almost effortless.

A few points within the text could stand enlargement and clarification even though they do not detract markedly from its value. For instance, little of the epidemiologic information, accumulated during the war, concerning *Streptococcus*, is incorporated. Needed especially, is a more clear-cut concept of "non-suppurative" complications. One could also approve more definitive statements of dosage for certain drugs; too much leeway is given instead of expression in absolute terms of grains per pound or grams per kilo. The detailed discussions of supportive therapy are fascinating since the elaborate attention paid diet, baths, skin care, counterirritants, nasal douches, etc., is in contrast to usual practice in this country. However, he slides rather vaguely over the rôle of transfusions, parenteral fluids, accessory foods, plasma and other measures commonly used here in the treatment of infectious disease.

One mark can be scored for and one against the publisher. In the publisher's favor is the excellent choice and reproduction of photographs. This is most welcome since the trend of publishing houses has been toward the elimination of new black and white prints in favor of poorly done color plates or old-timers resurrected from earlier editions. The adverse mark is given for what must be an oversight; while the writer makes frequent reference to the literature by both author and year, there is, at least in my copy, no bibliography. Finally, this book can be recommended unreservedly to all planning the publication of tomes on clinical medicine. Doctor Joe's forbearance in delaying contribution until his personal experience reached its peak, his refusal to discuss conditions with which he has not had intimate association, and his critical selection of references, all are criteria that might be followed by others with profit to self and reader.

Detroit

PAUL V. WOOLLEY, JR.

Medicine in the Changing Order. Report of the New York Academy of Medicine Committee on Medicine and the Changing Order. 240 pp. \$2.00. New York: The Commonwealth Fund, 1947.

This concise report covers the results of the work of an outstanding committee of the New York Academy of Medicine under the chairmanship of Dr. Malcolm Goodridge. Thirty-three physicians and 17 representatives of allied professions and lay interests served on the committee. In addition to presenting an extensive review of literature and statistical material, the information and opinions expressed before the committee and its subcommittees by some 50 persons are presented.

The origins of the present problems in American Medicine are given by way of introduction. The great improvement in the nation's health which has occurred since the beginning of our early history is recorded, but it is frankly admitted that there is a definite failure to make the most of present medical knowledge because of an uneven distribution of facilities. The contrast of medical care in urban areas with rural areas is drawn, as well as an analysis of the quality of medical care now available. A plea for greater inclusion of preventive medicine in the training and practice of the physician is made. The rôle of the diagnostic clinic, the hospital and allied professions, such as nursing, is well brought out.

Finally, prepayment insurance, both voluntary and compulsory, is analyzed and the conclusion drawn that many of the problems facing medicine today can be remedied by adopting voluntary insurance plans that may vary in scope in different areas, but which will, by slow and steady progress, attain the goal of more even distribution of medical care.

The material is nicely presented and contains a well conceived plan for medicine's adjustment to the changing order. All physicians will find this small volume well worth reading.

Detroit

BRUCE H. DOUGLAS

AN EVALUATION OF METHODS FOR SERUM PROTEINS*

THEODORE J. BERRY, M.D., AND ELLA PERKINS, M.S.

*From The Clinical Laboratories, Jefferson Medical College Hospital,
Philadelphia, Pennsylvania*

According to the majority of investigators, the normal serum protein concentration is said to be between 6.0 and 8.0 gm. per 100 ml. In our laboratory, it was observed that the stated normal range was considerably higher than our values. To reconcile the discrepancy, we undertook (1) to check the method by which we determine blood protein levels and to compare it with some of the other recognized biochemical technics, and (2) to calculate the average normal serum protein concentration from results obtained in 327 consecutive blood donors.

CHEMICAL METHODS COMPARED

In the selection of a biochemical technic for routine use in an active hospital laboratory, the factors of speed, facility and accuracy of method are the criteria of applicability. Since this evaluation is purely for clinical purposes, only a few of the methods will be discussed, and the tedious, complicated, time-consuming procedures of the laboratories of chemical research will not be considered.

1. *The Turbidimetric Method*

The determination of serum protein concentration is made in this laboratory by the turbidimetric method of Looney and Walsh^{6,7} with a slight modification necessary for its use with the Evelyn photo-electric colorimeter. A 1 to 100 dilution of serum in 15 per cent sodium chloride is prepared, of which 4 ml. is added to 1 ml. of a 2 per cent gum ghatti solution, followed by 5 ml. of a 5 per cent sulfosalicylic acid solution. After ten minutes, the resulting turbid suspension is read by means of the photo-electric cell, through a #520 filter, and the total serum protein is calculated by comparison with a curve standardized by the Kjeldahl method.

Although it has received widespread acceptance as the best single method of total nitrogen determination, the basic Kjeldahl procedure is said to be liable to errors of at least 10 per cent. The grosser discrepancies are considered to be due to the particular Kjeldahl variation used.^{2a} Obviously, so cumbersome a technic as the Kjeldahl is impractical for routine use in a busy biochemical laboratory of a hospital, such as ours, where an average of 4500 various chemical procedures are executed in one month. In this study, therefore, it was utilized only to standardize our test serums.

2. *The Micro-Kjeldahl Technic*

In Table 1, a comparison is made between the micro-Kjeldahl (Howe modification)² and the turbidimetric methods of protein determination in a split, pooled sample of serum.

* Received for publication, July 21, 1947.

For lack of a better term, we have used the expression "inherent error" to indicate the factor of variability within a given biochemical technic, or the scatter of values obtained on executing multiple determinations on the same split specimen. This was calculated in the following manner. Several samples of a pooled quantity of blood were sent to the laboratory under different names, and the average of the values, as reported by the technician, was calculated. The variation from the mean was expressed as a per cent, and called the "inherent error". This error comprises a number of factors necessarily taken into account in the evaluation of a clinical laboratory method. It affords an approximate arithmetic estimate of personal and technical faults which may vary considerably with the procedure in question, the skill of the executor, and the integrity of the mechanical elements involved. In our estimate of this error, the latter two factors were

TABLE 1
COMPARISON OF TURBIDIMETRIC AND MICRO-KJELDAHL METHODS OF
PROTEIN DETERMINATION

NUMBER	TURBIDIMETRIC METHOD					MICRO-KJELDAHL METHOD				
	Total protein gm. per 100 ml.	Differ- ence	Inherent error	Macro-Kjeldahl		Total protein gm. per 100 ml.	Differ- ence	Inherent error	Macro-Kjeldahl	
				Error	Per cent error				Error	Per cent error
1	5.3	.04	0.75	.26	4.67	4.23	0.72	14.4	1.33	23.92
2	5.3	.04	0.75	.26	4.67	4.71	0.24	4.8	0.85	15.28
3	5.1	.16	3.13	.46	8.24	5.18	0.23	4.6	0.38	6.83
4	5.1	.16	3.13	.46	8.24	5.24	0.29	5.8	0.32	5.77
5	5.2	.06	1.15	.36	6.47	5.24	0.29	5.8	0.32	5.77
6	5.3	.04	0.75	.26	4.67	4.23	0.72	14.4	1.33	23.92
7	5.4	.14	2.59	.16	2.87	5.46	0.51	10.2	1.10	1.79
8	5.3	.04	0.75	.26	4.67	5.18	0.23	4.6	0.38	6.83
9	5.3	.04	0.75	.26	4.67	4.82	0.13	2.6	0.64	11.54
10	5.3	.04	0.75	.26	4.67	5.25	0.30	6.0	0.31	5.55
Averages . . .	5.26	.07	1.45	.30	5.38	4.95	0.36	7.32	0.596	10.72

virtually constant throughout the investigation. Theoretically then, the variation under these circumstances should be negligible, but such mathematical precision is not generally observed in routine performance.

To calculate the "inherent error", ten determinations were made on the split serum by each method (turbidimetric and the micro-Kjeldahl), and to establish the approximate error of the individual technic, the values were compared with those obtained by the macro-Kjeldahl determination. It was found that the "inherent error" in the turbidimetric method was 1.45 per cent, as compared with 7.32 per cent in the micro-Kjeldahl technic.² The error of the former was 5.38 per cent, and with the micro-Kjeldahl determination, the error was 10.72 per cent.

As a further check on the turbidimetric method, it was compared with a standard serum prepared for a state survey by the William Pepper Laboratory of the

University of Pennsylvania. The readings obtained by the tested technic were less than 3 per cent in error.

The micro-Kjeldahl modifications are more tedious, exacting, and time-consuming, and these figures indicate that it is subject to wider variations than the method of Looney and Walsh.

3. Gradient Methods

(a) *The specific gravity correlation method.* The method of determining the blood protein concentration by correlation of the specific gravity of the serum or plasma, as outlined by Phillips and Van Slyke⁸ is perhaps the simplest and quickest quantitative method available. It is, however, subject to great error, almost always giving a reading well in excess of the true value, as shown by Hoch and Marrack,^{2a} and others.¹ In studying the graph (Fig. 1) which represents over 100 consecutive, parallel serum protein determinations by both the turbidimetric and the Phillips-Van Slyke specific gravity methods, it is apparent that no real, sufficiently accurate correlation exists. The difference reaches as high as 48.2 per cent, with a mean difference of 16.2 per cent.

In a group of twelve patients with the same serum protein concentration (5.4 gm. per 100 ml.) as calculated by the turbidimetric method, the specific gravities of the serums varied between 1.0230 to 1.0270, or, when converted to the protein equivalent, the values ranged from 5.90 to 7.45 gm. per 100 ml. The higher reading represents a difference of 35.4 per cent.

Serums from 16 patients, exhibiting the same specific gravity (1.0250) were found to have serum protein concentrations ranging from 4.5 gm. per 100 ml. to 6.4 gm. per 100 ml. The greater reading represented a difference of over 48 per cent from the calculated total protein concentration of that particular sample.

(b) *The falling drop method* The method of Kagan³ enables one to determine the specific gravity of a single drop of blood by comparing its time of fall through a liquid of known specific gravity, and referring this information to an alignment chart. The serum protein is calculated by using any one of a number of formulas of conversion. The formula used by Kagan differs from that used by Phillips and Van Slyke in the copper sulfate method, in that it usually gives slightly lower protein correlations.

These methods rely on the premise that the protein content of blood serum is the only significant factor in the alteration of the specific gravity. From observations in studies of specific gravity-protein content relationship, it would seem that factors other than the protein content of body fluids, such as salts and fats, affect the specific gravity, and these do not follow the proposed formulas and conversions factors with any practical degree of accuracy.

4. The Biuret Reaction

Direct comparison with the biuret reaction⁴ was not undertaken in this investigation. This test is believed by many to be thoroughly reliable, the error usually not exceeding 5 per cent in experienced hands.

THE NORMAL SERUM PROTEIN CONCENTRATION

Three hundred and twenty-seven consecutive blood donors were used as the control group to determine the normal adult serum protein concentration. The

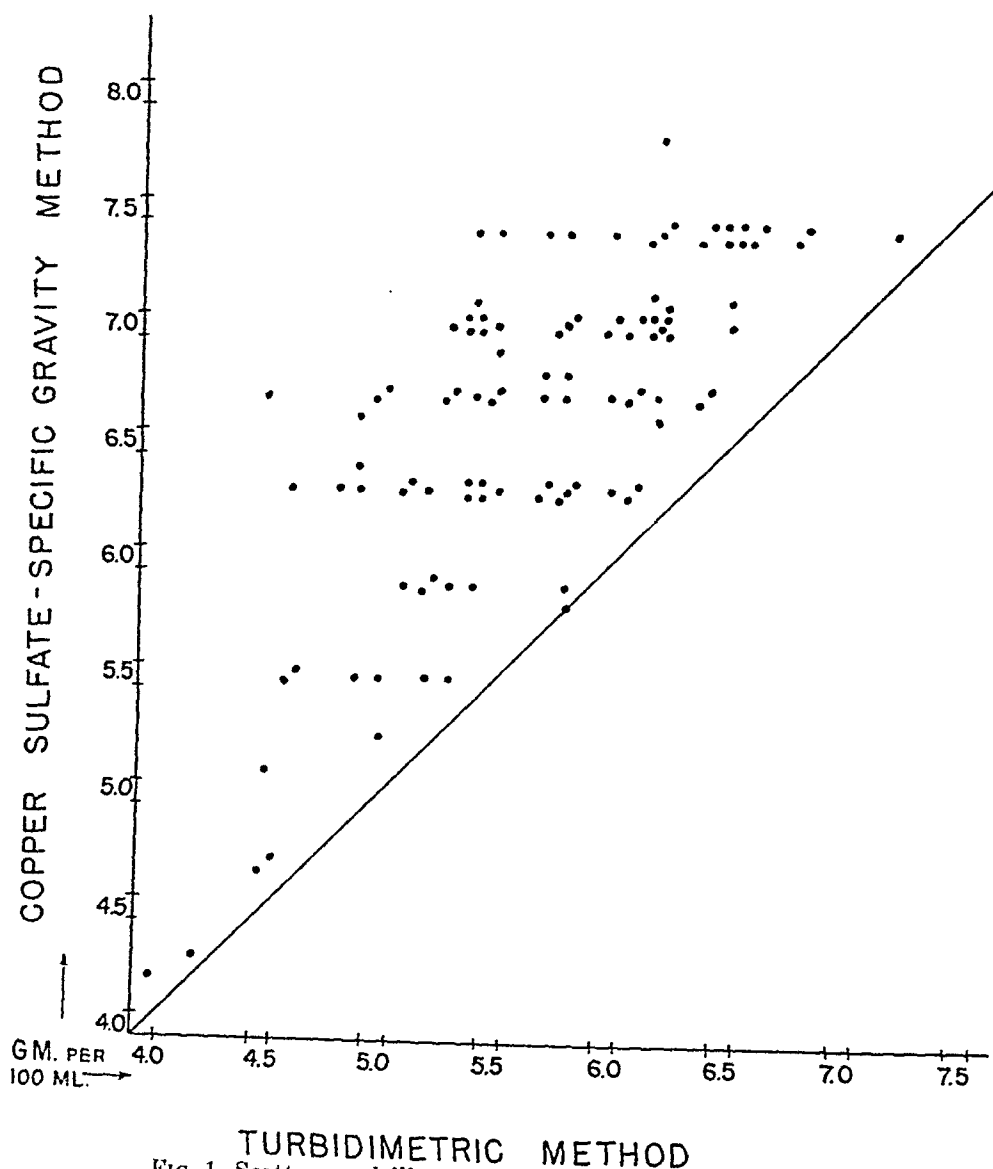


Fig. 1. Scatter graph illustrating the correlation between the turbidimetric and the specific gravity methods of serum protein determination.

results were obtained during the first two months of 1947. Figure 2 is a graphic demonstration of the protein values obtained in this group. The range is from 5.2 gm. per 100 ml. to 7.7 gm. per 100 ml., with a mean of 6.33 gm. per 100 ml. Only 3.1 per cent were above 7.0 gm. per 100 ml., and 8.2 per cent were below 6.0 gm. per 100 ml. The average is slightly lower than that generally set forth by

other observers, and can be accounted for in part by the fact that there is statistical evidence of a generalized diminution in the blood protein values among the hospitalized patients during the latter years of the recent war. The average

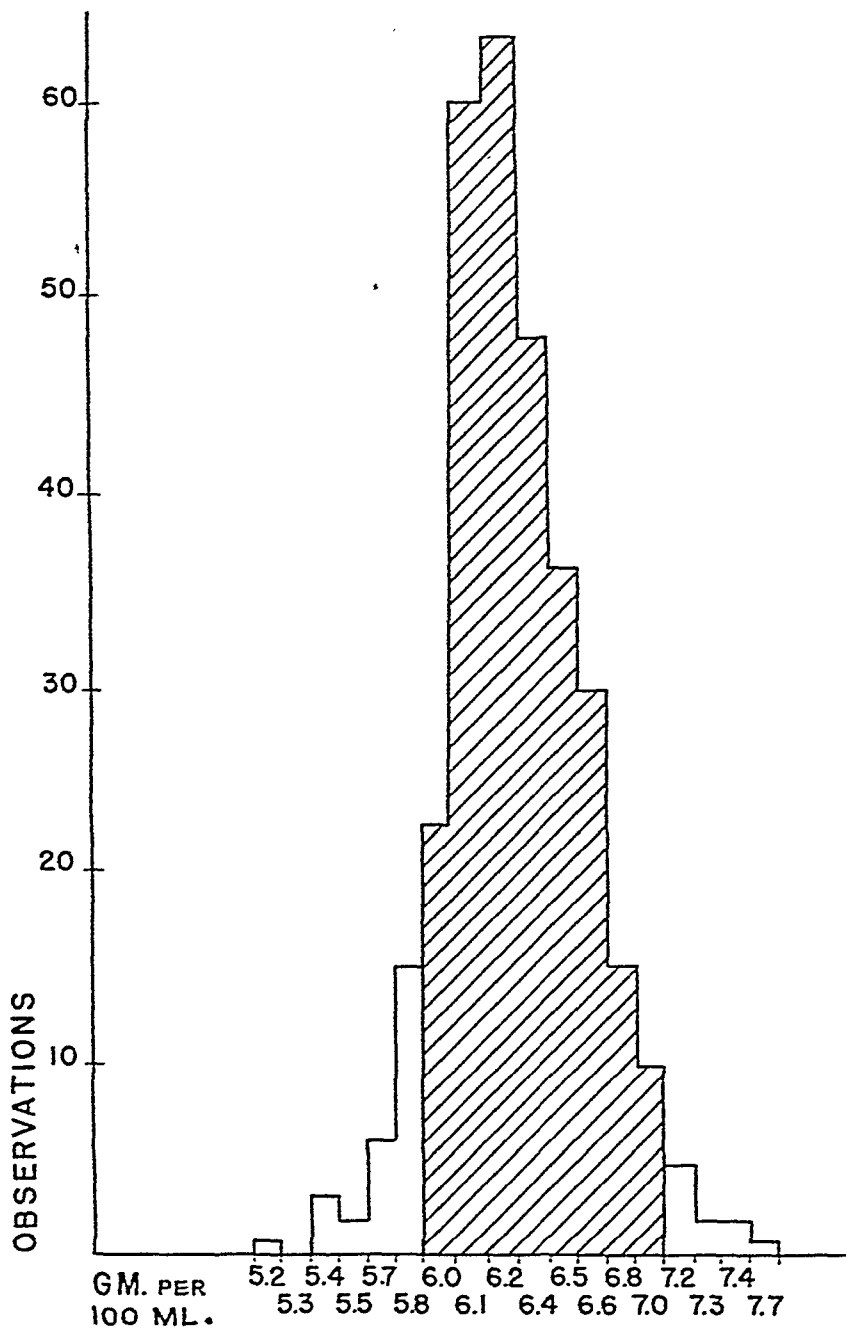


FIG. 2. Graphic representation of the serum protein concentration in 327 blood donors.

serum protein concentration of the hospitalized patients began its gradual decline in 1944 and reached its lowest level in the summer of 1946, when the mean concentration for all patients, on whom the test was requested by the attending staff, was 5.5 gm. per 100 ml. Coincident with a more plentiful supply of pro-

tein foods in the autumn of 1946, there began a gradual rise in the average level among patients of the same type until the present time when the mean is approximately 6.0 gm. per 100 ml. A study of more than 1300 blood protein determinations since the early part of World War II, in patients from this hospital, revealed this striking fall of the protein level, and the subsequent rise following the return of a better nutritional status among this urban population.

CONCLUSIONS

1. The turbidimetric method of Looney and Walsh is a suitable biochemical technic for use in a clinical laboratory of a hospital for the determination of the blood protein concentration. Its "inherent error" is less than 1.5 per cent and its error does not exceed 6 per cent.

2. The estimated error in the micro-Kjeldahl method of protein determination is 10.7 per cent, and its "inherent error" is more than 7 per cent. Because of the exacting technic of this method and its relative difficulty in execution, its use as a routine procedure is not recommended.

3. The copper sulfate method for determining plasma and serum specific gravities and correlating the protein values is liable to errors up to 48 per cent and is unsuitable for routine clinical laboratory use.

4. The range of serum protein concentrations in 327 consecutive blood donors was from 5.2 gm. per 100 ml. to 7.7 gm. per 100 ml., with a mean of 6.33 gm. per 100 ml., and a standard deviation of 0.58 gm. per 100 ml.

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A SURVEY OF THE ACCURACY OF CHEMICAL ANALYSES IN CLINICAL LABORATORIES*

WILLIAM P. BELK, M.D.,† AND F. WILLIAM SUNDERMAN, M.D.‡

In 1946 the Committee on Laboratories of the Medical Society of the State of Pennsylvania proposed a survey‡ to check the accuracy of some of the more common chemical measurements made in hospital laboratories throughout the state. It undertook to do this by distributing solutions which had been carefully

TABLE 1
NUMBER OF DETERMINATIONS CLASSED AS SATISFACTORY, UNSATISFACTORY
AND GROSS ERROR
September Analyses

SUBSTANCE TESTED	SATISFACTORY LIMITS OF RESULTS PER 100 ML.	NUMBER SATISFACTORY	NUMBER UN- SATISFACTORY**	GROSS ERROR**
Hemoglobin.....	9.8 ± 0.3 gm.	17	34	11
Hemoglobin.....	15.1 ± 0.5 gm.	21	31	3
Glucose.....	60 ± 10 mg.	33	19	5
Glucose.....	375 ± 30 mg.	27	24	4
Sodium chloride.....	456 ± 50 mg.	30	14	2
Total protein.....	6.6 ± 0.4 gm.	18	29	7
Albumin.....	4.6 ± 0.3 gm.	9	35	7

October Analyses

SUBSTANCE TESTED	SATISFACTORY LIMITS OF RESULTS MG. PER 100 ML.	NUMBER SATISFACTORY	NUMBER UN- SATISFACTORY**	GROSS ERROR**
Calcium.....	6.6 ± 0.5	14	29	12
Calcium.....	12.6 ± 0.5	14	30	11
Urea nitrogen.....	45 ± 5	14	27	13
Urea nitrogen.....	9 ± 1	21	21	14
Sodium chloride.....	642 ± 50	28	15	2
Sodium chloride.....	447 ± 50	28	15	1

** The number unsatisfactory includes the gross error. Thus, of the 34 unsatisfactory hemoglobin determinations, 11 made errors of 1.5 or more gm. per 100 ml.

prepared for analyses. A letter was sent to the pathologists and clinical pathologists in the state explaining the purpose of the survey and 59 expressed their

* The cost of the original survey was defrayed by the Medical Society of the State of Pennsylvania; that of the follow-up survey, by the Pennsylvania Association of Clinical Pathologists. Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 28, 1947. Received for publication, July 21, 1947.

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‡ The Committee consisted of Henry F. Hunt, M.D., George R. Lacy, M.D., Verner Nisbet, M.D., Lloyd E. Wurster, M.D., and William P. Belk, M.D., chairman. F. William Sunderman, M.D., served as referee for this survey.

willingness to participate. These physicians represented hospitals of all types throughout Pennsylvania as well as two hospitals in New Jersey, one in Delaware and a small number of private laboratories.

Each participant received a total of 12 samples for analyses including 2 aqueous solutions of glucose, 3 of chloride, 2 of urea, and 2 of calcium in concentrations comparable to those found in human blood; one sample of serum for measurements of total protein, albumin and globulin; and 2 samples of citrated whole blood for the measurements of hemoglobin concentration. The aqueous solu-

TABLE 2
FREQUENCY DISTRIBUTION OF LABORATORY SCORES
(Score = Total Number of "Satisfactory" Determinations)
September Data

SCORE (MAXIMUM POSSIBLE = 7)	NUMBER OF LABORATORIES HAVING THIS SCORE
0	0
1	9
2	15
3	8
4	11
5	6
6	3
7	0

October Data

SCORE (MAXIMUM POSSIBLE = 6)	NUMBER OF LABORATORIES HAVING THIS SCORE
0	6
1	6
2	6
3	9
4	13
5	2
6	2

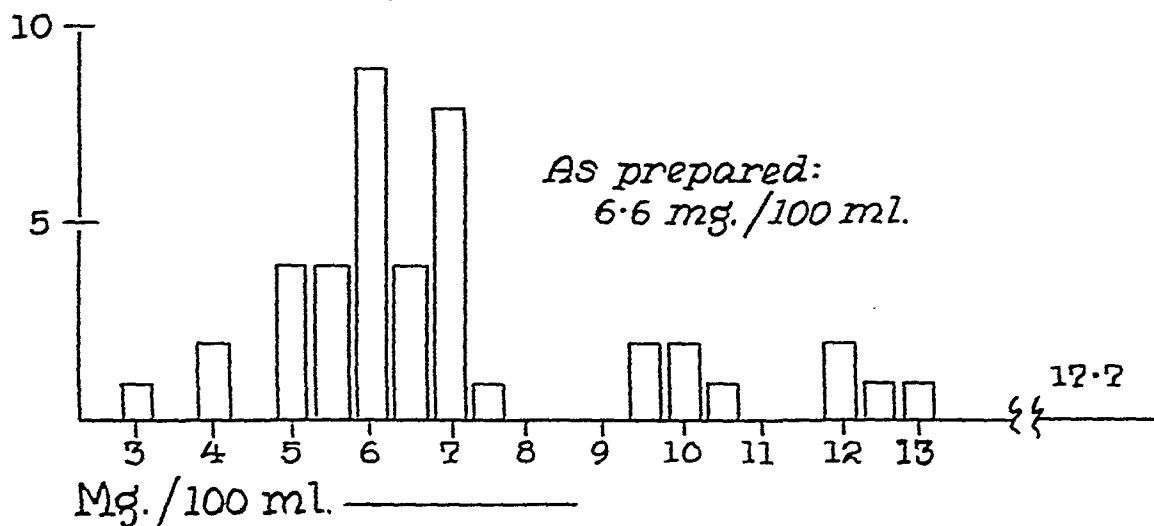
tions, serum and whole blood samples were found upon analysis by the referee to have remained unchanged during transit. For the sake of convenience the samples were sent to the participants in two sets on different occasions. These sets are designated "September analyses" and "October analyses" in the tables. The results of the analyses were returned to the chairman of the committee on unsigned, uniformly printed postcards so that the work of any participant could not be identified.

The data were grouped into classes and arranged into frequency distributions. Histograms were then prepared as shown in Figures 1 to 6 inclusive. The number of satisfactory and unsatisfactory results and gross errors are listed in

Table 1. The allowable errors for the various types of measurements are given in column 1, such values having been arbitrarily selected by the referee as being limits that should be maintained for satisfactory laboratory practice (Table 2).

CALCIUM

Frequency



Frequency

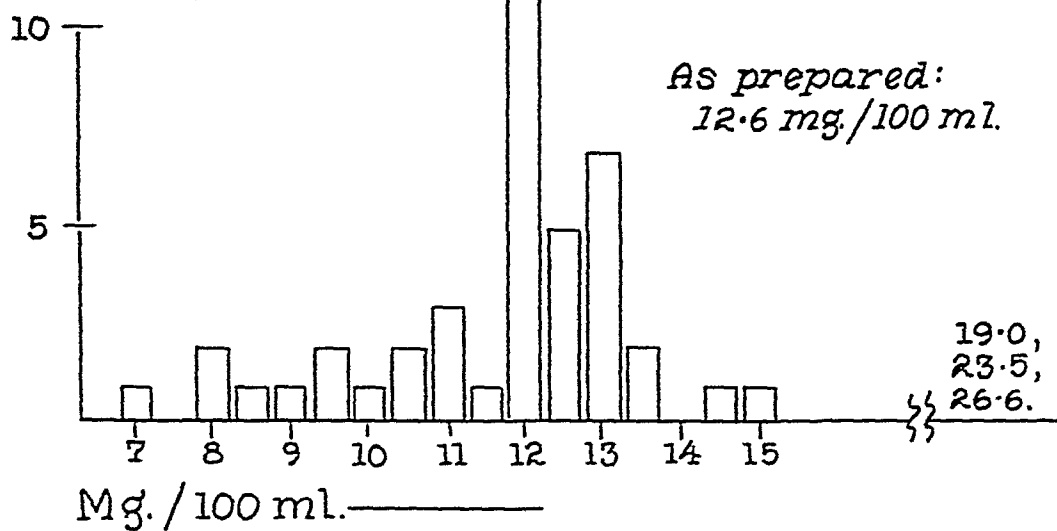


FIG. 1

The data presented in the tables and figures are self-explanatory. They should, however, be carefully scrutinized so that their full implications will not be missed. The scatter of the measurements and the degree of unreliability is surprising. The accuracy of the measurements is below any reasonable standard.

It will be noted that unsatisfactory results outnumbered the satisfactory and that no laboratory had a perfect score.

The Pennsylvania Association of Clinical Pathologists followed up this survey with a questionnaire to the clinical pathologists of the state requesting their

UREA

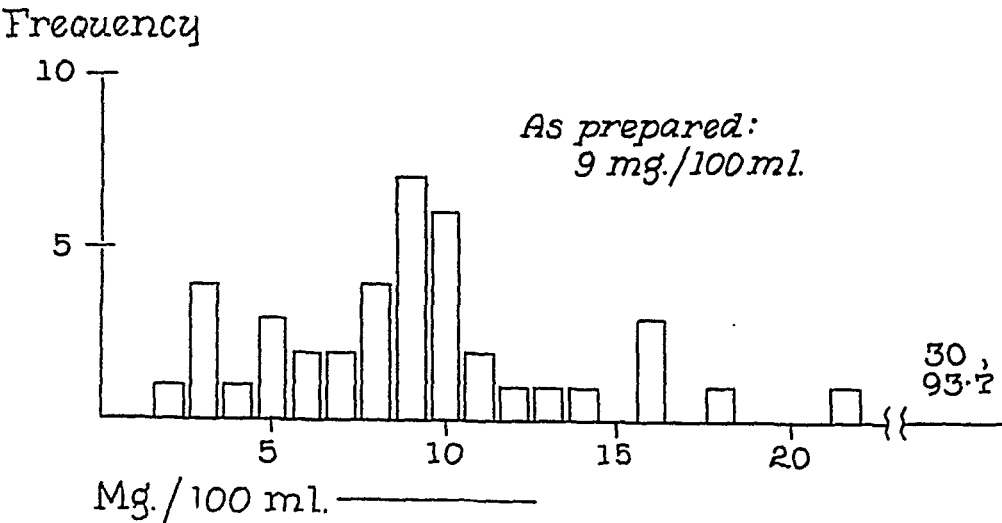
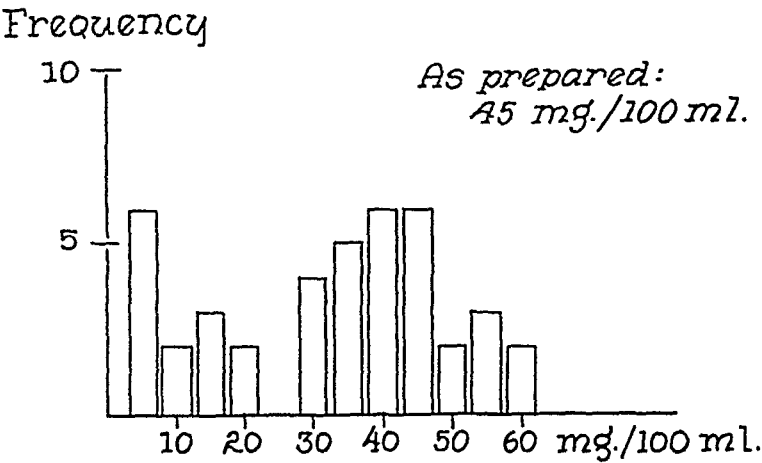


FIG. 2

opinions of the causes of the inferior laboratory work indicated by the survey. One hundred and six replied, 95 of whom listed the factors that they considered important, as shown in Table 3.

It will be seen in Table 3 that 82 of the laboratory directors indicated that in their opinions inadequate training of technicians was a major contributing factor.

SODIUM CHLORIDE

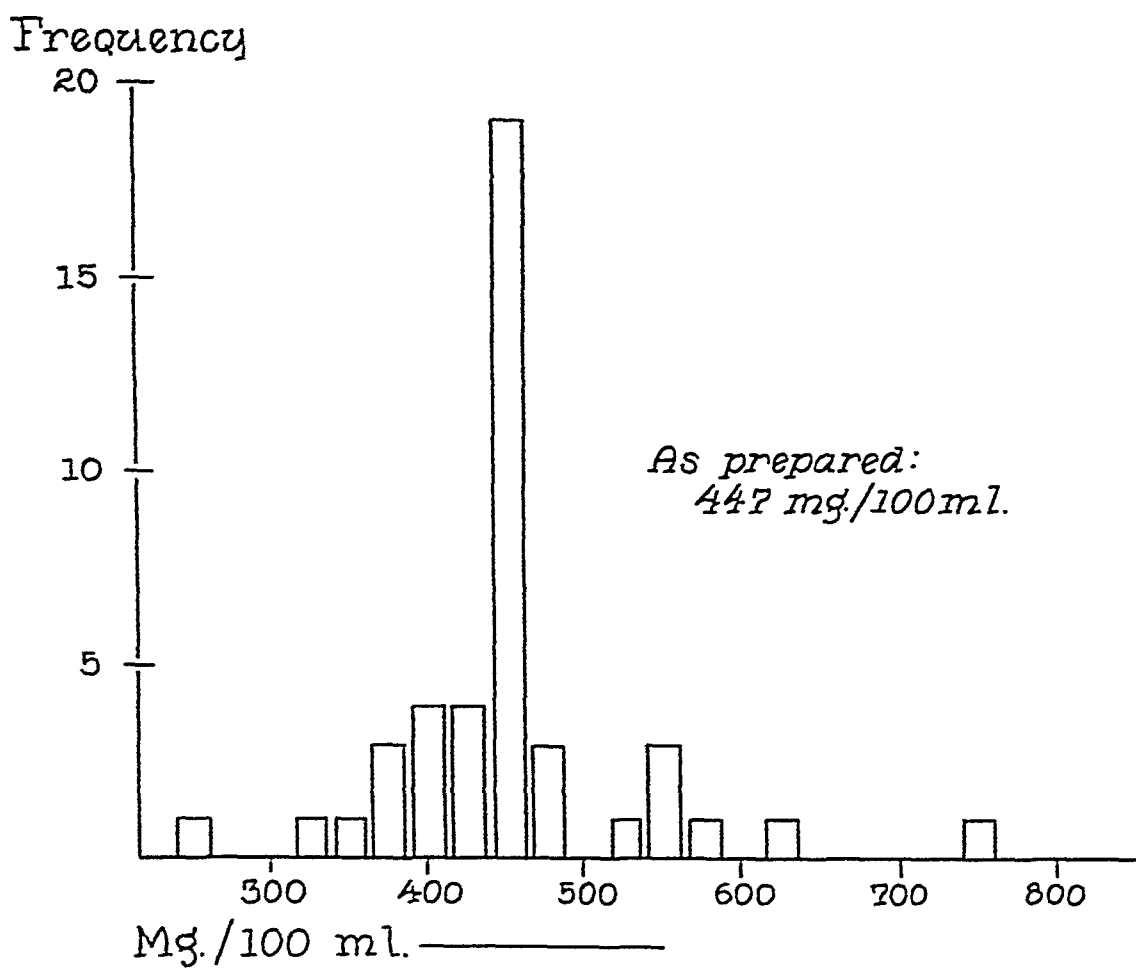
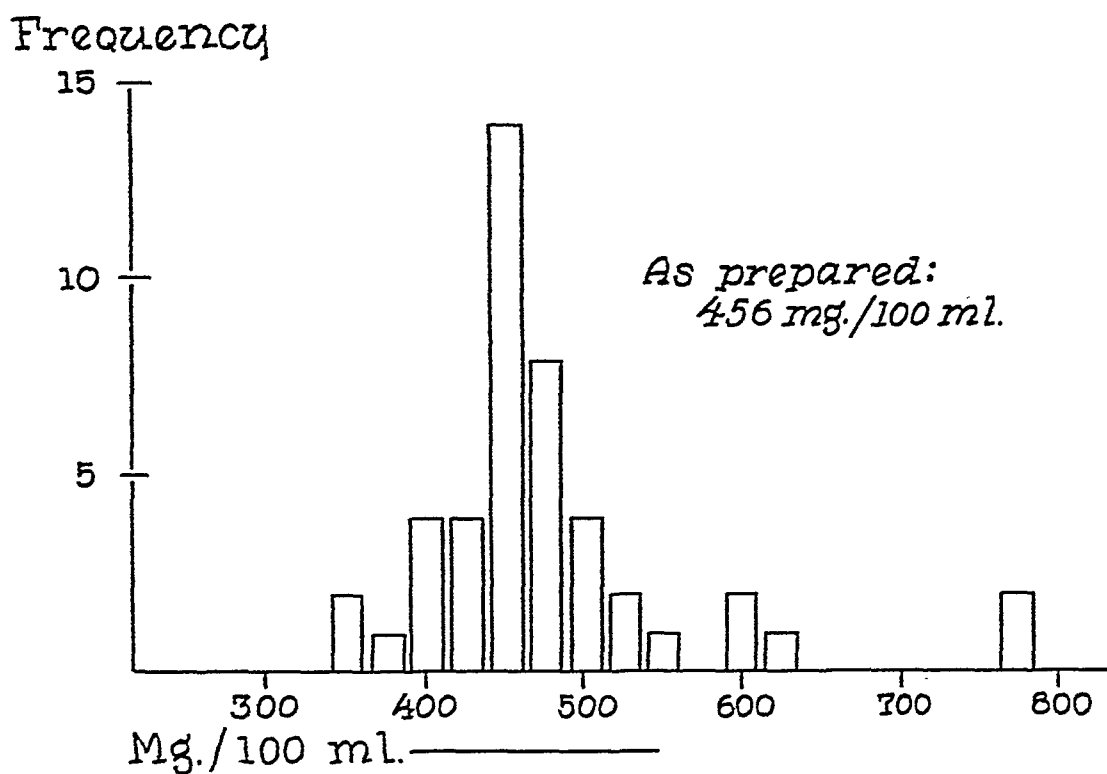


FIG. 3

HEMOGLOBIN

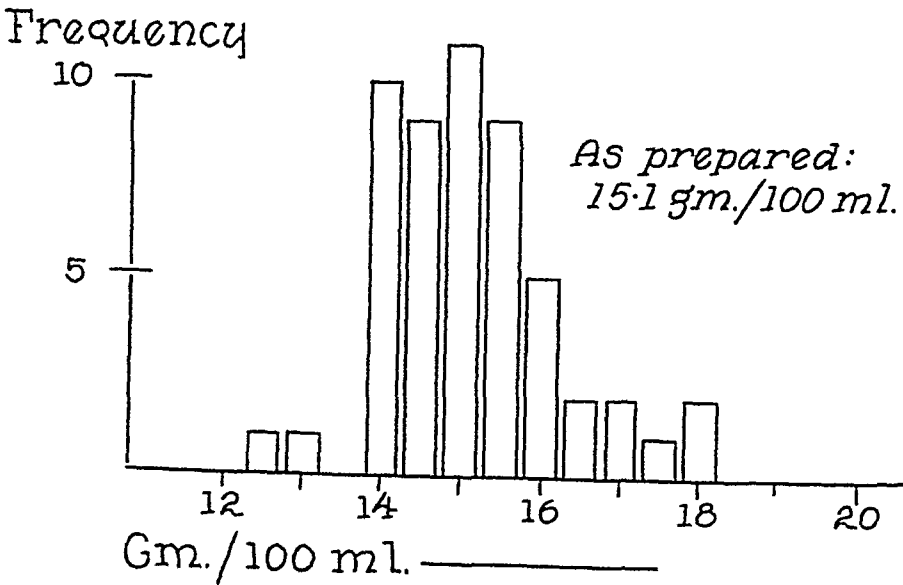
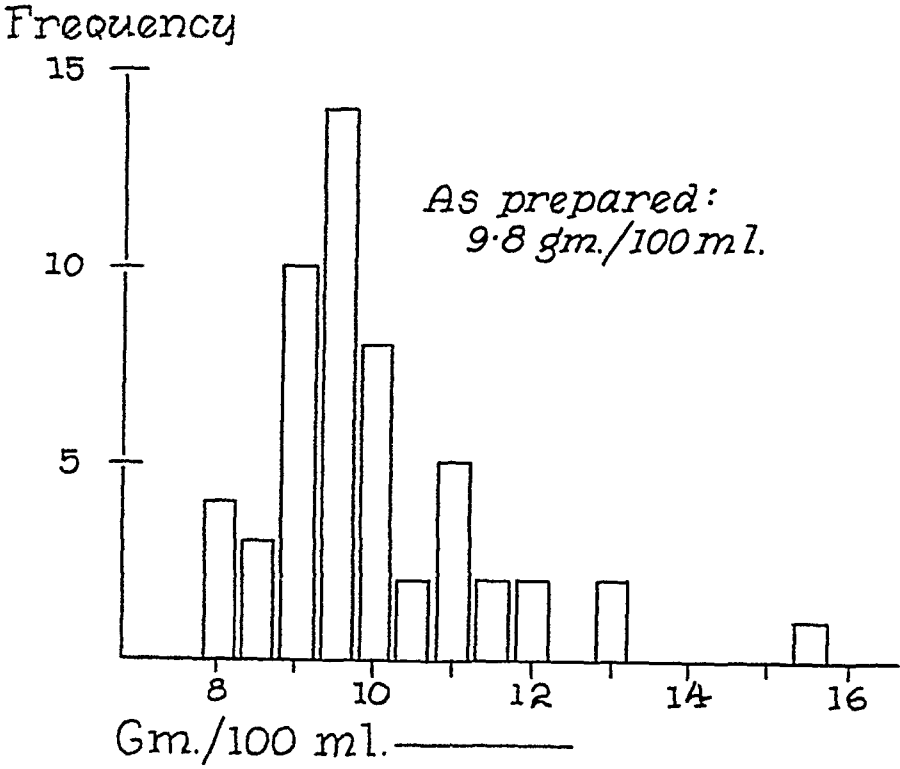


FIG. 4

In addition, 80 felt that an insufficient number of technicians was provided to carry out the amount of work undertaken by the laboratories. It is recognized

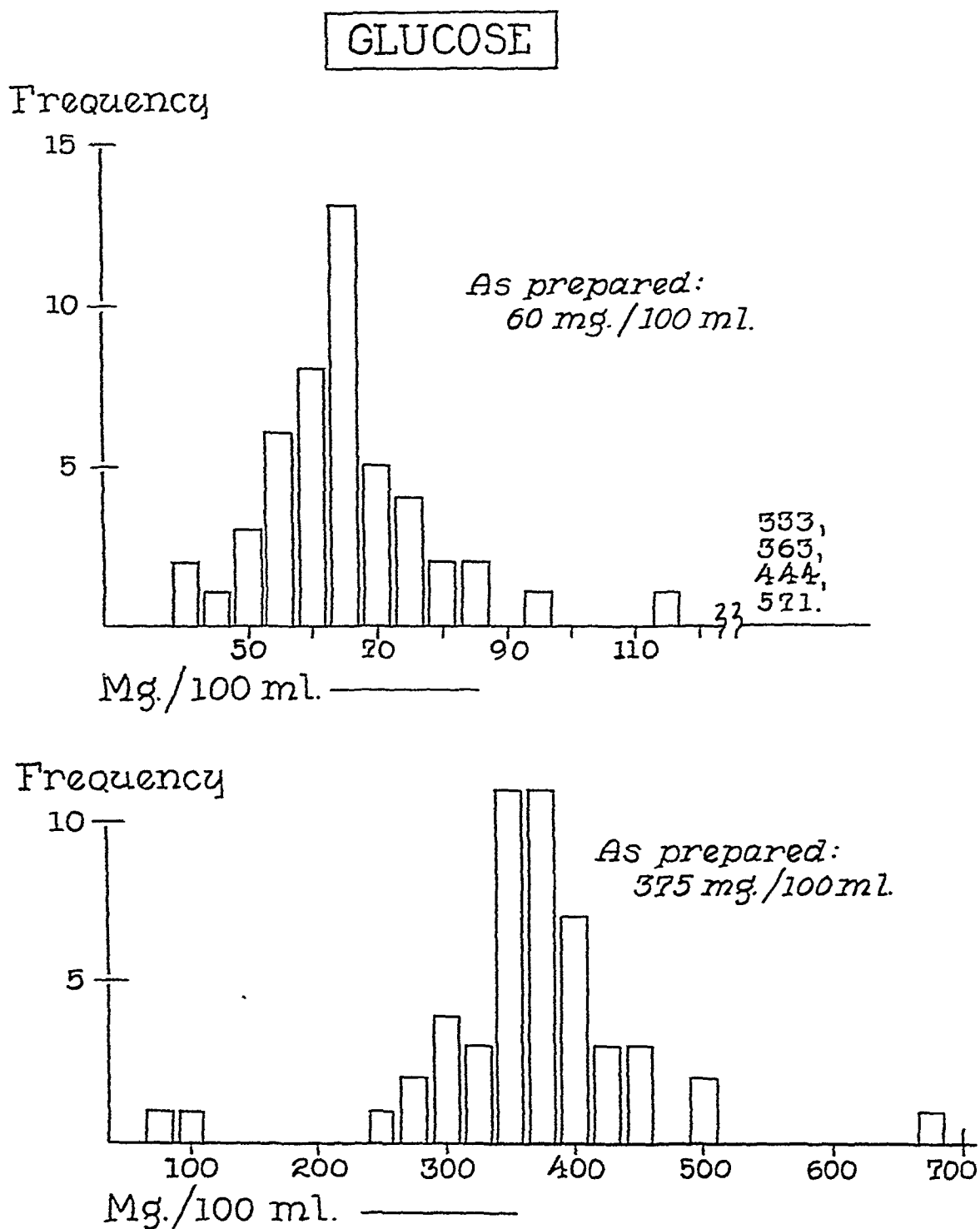


FIG. 5

that the most capable technicians cannot be expected to obtain reliable results when placed under pressure to perform an excessive number of examinations

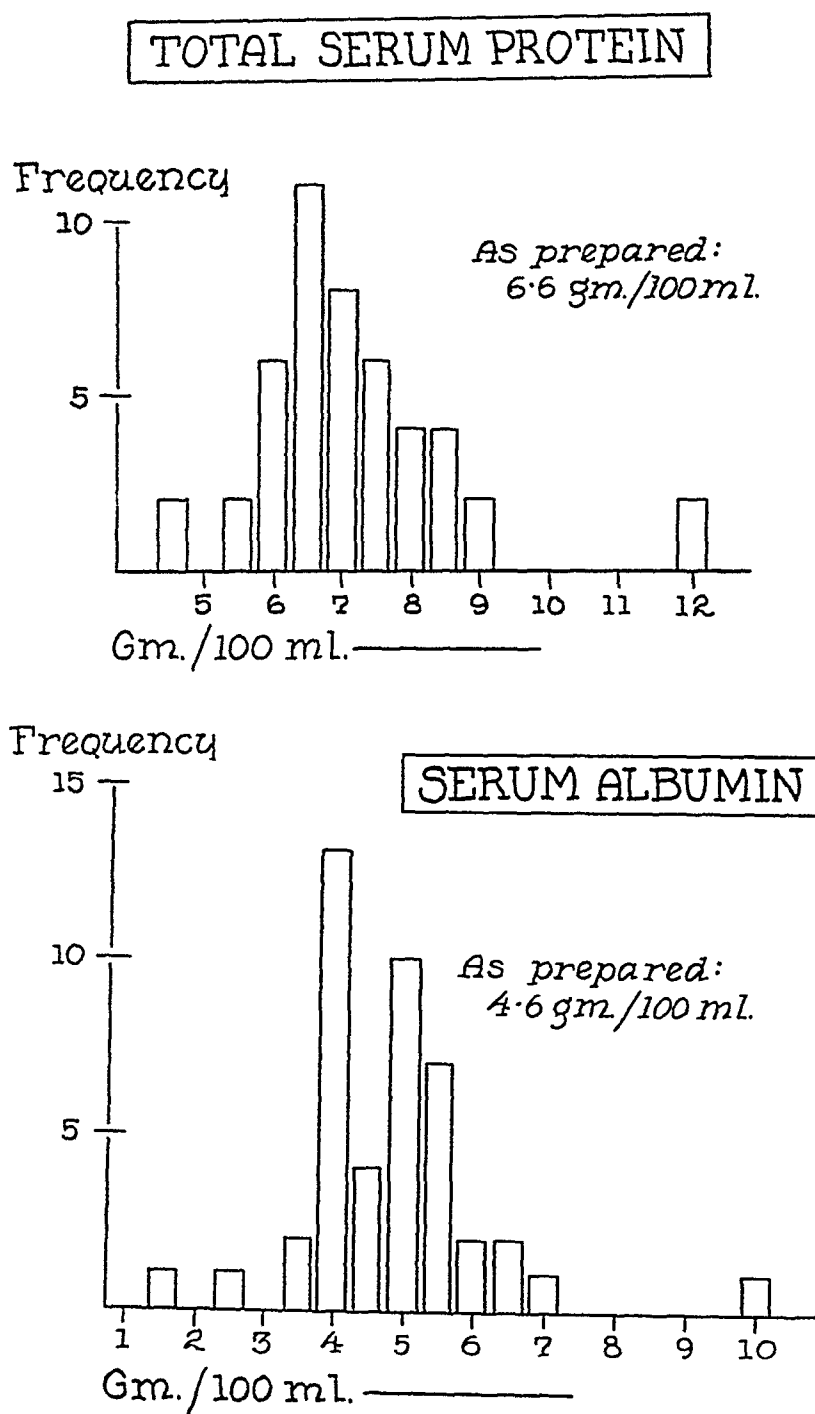


FIG. 6

within a limited period of time. From the replies to the questionnaires it was apparent that the majority of the pathologists felt that the combination of poor

training and insufficient number of technicians accounted in large part for the unreliability of many of the analyses reported in the survey.

Technician training and supervision are obviously responsibilities of the clinical pathologist. At the present time, however, he is likely to encounter obstacles in fulfilling these obligations. In recent years it has been difficult to recruit apprentice technicians with satisfactory educational backgrounds, largely because prospective apprentices were able to secure equal or better remuneration in other occupations, some of which did not require prolonged preliminary training. In addition, the insufficient number of technicians is in part attributable to the unwillingness of many institutions to create new technical positions to meet the expanding needs of the laboratory, and also to the practice

TABLE 3
FACTORS CONTRIBUTING TO TECHNICAL INACCURACIES AND OTHER UNSATISFACTORY
CONDITIONS IN CLINICAL LABORATORIES
(Opinions of Ninety-five Pathologists in Pennsylvania)

FACTORS	TIMES LISTED
1. Poorly trained technicians.....	82
2. Inadequate number of technicians.....	80
3. Lack of understanding between pathologist and staff.....	64
4. Poor equipment.....	63
5. Insufficient floor space.....	57
6. Miscellaneous factors (inadequate orderly service, insufficient secretarial service, etc.).....	39

of fixing salaries at levels that are too low to attract and hold the better type of worker.

Item 3, "lack of understanding between pathologist and staff", is a reflection of the fact, generally recognized, that there is in present practice a hiatus between the laboratory and the ward. It may be necessary for the clinical pathologist, himself, to study the patient in order to meet fully the clinical needs.

SUMMARY

Results are reported of a survey which was conducted to check the accuracy of some of the more common chemical measurements made in hospital laboratories throughout Pennsylvania.

Acknowledgment. We are indebted to Dr. Charles P. Winsor of the Department of Biostatistics of Johns Hopkins University for the statistical analysis of these data.

DETECTION OF INTRAVASCULAR CLOTTING TENDENCY BY HEPARIN TOLERANCE PRINCIPLE

METHODS AND CLINICAL APPLICATION*

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The studies of De Takats¹ demonstrated an increased tolerance to intravenous heparin in cases of thromboembolic disease. Waugh and Ruddick^{3, 4} developed a test to measure increased tolerance to heparin *in vitro*. Ogura, Fetter, Blankenhorn and Glueck² further simplified the Waugh-Ruddick test by using only one tube. They demonstrated increased tolerance to heparin in 21 out of 27 cases of myocardial infarction. Owing to interest stimulated by the increasing use of anticoagulant therapy, a need was felt for a simple laboratory test which would aid in the diagnosis of an intravascular clotting tendency. The modified Waugh-Ruddick test was adopted and it is our purpose in this paper to present materials and methods for the performance of tests both *in vivo* and *in vitro* and the clinical applications for these tests.

MATERIALS AND METHODS

The original De Takats test involved three determinations of coagulation times at ten minute intervals following the intravenous injection of 10 mg. of heparin, so that thirty minutes elapsed for the performance of the test. Since his work showed that the first ten minute reading was the most significant, the test was initially modified by omitting all subsequent determinations. The normal response to this amount of heparin was a two- or three-fold increase in the coagulation time in ten minutes, with normal values in the twenty and thirty minute readings. Patients with thromboembolic disease, however, showed no response either in the first ten minute reading or in subsequent readings. The De Takats test, then, requires three venipunctures: determination of the control clotting time, injection of heparin and determination of the clotting time after ten minutes. In the determination of coagulation time, the capillary tube method gave inconsistent results and was therefore abandoned in favor of the Lee-White clotting time method.

The method of Waugh and Ruddick involved the use of increasing amounts of heparin added to venous blood *in vitro*. The original test utilized 0.1 Toronto unit of heparin with increments of from 0.1 unit to 0.7 unit. Ogura and his associates observed that clockwise rotation on a line chart of the clotting times by this method was always the result of acceleration in the last tube. They, therefore, depended on the 0.7 unit tube for their readings. Thus, the test was extremely simple and could be accomplished in the hospital laboratory. In our work, the heparin concentration used was converted to terms of milligrams and the test planned on that basis.

Stock heparin solution was prepared by diluting 1.0 cc. containing 10 mg. of

* Received for publication, July 17, 1947.

heparin with 99 cc. physiologic saline. One-tenth cc. of this material was placed into each of several scrupulously clean 10 mm. Kahn tubes and the tubes plugged with rubber stoppers. The stock material was stable, but was kept in the refrigerator in order to reduce bacterial growth, since sterile precautions were not observed. The test *in vitro* required 1.0 cc. of venous blood, removed as soon as possible following application of the tourniquet. This was mixed with the dilute heparin in the tube by inversion. Thus, a final concentration of 0.01 mg. of heparin added to 1.0 cc. of venous blood was obtained. The added heparin was kept at 0.1 cc. instead of 1.0 cc. as in the Waugh-Ruddick test to reduce the effect of dilution on the venous clotting time.

Each tube was observed for clotting at five minute intervals at room temperature. Merely tilting the tube to note the point where the fluid no longer ran along the side was not considered sufficient evidence of clotting. In many early tests, clotting occurred in the red blood cell layer and not in the plasma portion when the sedimentation rate was rapid. Therefore, it was necessary to invert the tube completely in order to determine the end point which was reached when the tube could be completely inverted without flow. Care was taken to avoid shaking or any other procedure which might produce bubbles on top of the fluid. If a thin fibrin coat formed on top of the fluid and was broken up by inversion, complete clotting followed in two or three minutes. Thus, no inaccuracies resulted from inversion.

Standardization of the delay in clotting produced by this concentration of heparin was accomplished by testing five normal subjects with each fresh batch. Some variation was encountered in the activity of heparin from vial to vial. The concentration of heparin in the final solution was then changed to conform with the activity noted. The selected concentration was intended to delay clotting a minimum of sixty minutes in the normal subject. As long as the test was controlled by standardization with normal blood serums, it could be run at room temperature. If standardization was not practiced, then seasonal changes in room temperature had an appreciable effect, as Whittaker⁵ has pointed out. The test, then, must be run at a selected standard water bath temperature.

BASIS FOR HEPARIN TOLERANCE TESTS

Waugh and Ruddick considered their test to be a measure of circulating thromboplastin. Acceleration in clotting time was produced by adding thromboplastin to the heparin blood mixture. Delay in clotting could be produced by removal of platelets by centrifugation.

Since the test was considered a measure of circulating thromboplastin, a patient having an excess of thrombocytes which break down readily should show increased tolerance to heparin. A case was encountered where these experimental conditions prevailed. A 32 year old white man, an army veteran, was admitted to the hospital because of arterial bleeding following the incision of a hematoma of the back. This patient had had a splenectomy two years previously because of trauma to the spleen. On admission, his platelet count was in excess of 3 million per cu. mm. After a heparinized blood sample from this patient had remained exposed to the air for thirty minutes, only one

million platelets per cu. mm. could be counted. Thus, large amounts of thromboplastin were continually being formed. The data in Table 1 show the increased tolerance to heparin by the method, both *in vivo* and *in vitro*. The negligible response to 10 mg. of heparin injected intravenously in the modified De Takats test and the accelerated reaction in the modified Waugh-Ruddick test *in vitro* point to neutralization of excess thromboplastin by the added heparin.

CLINICAL APPLICATION

In the work of De Takats, all cases of thromboembolic disease showed increased tolerance to heparin. With the test *in vitro*, it is possible to apply this principle to the study of intravascular clotting. There are many instances in which the diagnosis of pulmonary embolism is in doubt. This diagnosis is further ob-

TABLE 1

RESULTS OF HEPARIN TOLERANCE TESTS *in vivo* AND *in vitro* IN A CASE OF INCREASED THROMBOCYTE COUNT

	CONTROL	TEN MINUTES AFTER INTRAVENOUS INJECTION OF 10 MG. HEPARIN
<i>In vivo</i> test	7 minutes	10 minutes
<i>In vitro</i> test	14 minutes	14 minutes

TABLE 2

DISTRIBUTION OF THROMBOEMBOLIC DISEASES IN 25 PATIENTS HAVING HEPARIN TOLERANCE *in vitro* BELOW THIRTY MINUTES

DIAGNOSES	NUMBER OF PATIENTS
Phlebothrombosis	11
Acute thrombophlebitis.	5
Pulmonary thrombosis following septal myocardial infarction..	3
Pulmonary embolism, source unknown	1
Pulmonary embolism following phlebothrombosis.	3
Renal infarction from auricular fibrillation.....	1
Femoral artery embolism from myocardial infarction.....	1

scured when the occurrence of pulmonary embolism is accompanied by little or no subjective or objective evidence of phlebothrombosis. Other postoperative pulmonary complications are sometimes difficult to differentiate from embolism. With proper interpretation, the test *in vitro*, as well as the test *in vivo*, offers an aid to diagnosis. Negative results are of as great value as positive results, since no case of thromboembolic disease has so far been observed without increased tolerance to heparin.

Because the normal delay by the method *in vitro* is pegged at sixty minutes, minor accelerations which occur should be disregarded. In 25 patients, hospitalized for various conditions other than thromboembolic disease, results as low as forty minutes were encountered. Waugh and Ruddick showed that these results represent changes due to bed rest or infections. A result of less than thirty min-

utes in any given patient, however, is significant of a markedly increased tolerance to heparin.

Table 2 shows the types of cases represented in a series of 25 patients with thromboembolic disease. All of these patients showed heparin tolerance *in vitro* with clotting times of less than thirty minutes. Tests *in vivo* were run on 10 of these patients and likewise showed a lack of response to injected heparin.

A group of 25 patients with myocardial infarction was also studied. The results confirmed those obtained by Ogura *et al.* The clotting tendency as demonstrated by the heparin tolerance test *in vitro* became accelerated in 15 of the 25 patients in the second week of the onset. As in the series reported by Ogura *et al.*, acceleration occurred as early as the third day in 8 of the 15 patients. Three of this latter group developed thromboembolic complications and are included in the thromboembolic series. None of the patients who did not show accelerated reactions developed these complications. In many instances, the heparin tolerance tests indicated a clotting tendency but the patients never developed thromboembolic complications. How this clotting tendency reverts to normal spontaneously is still unknown.

From the results in the 25 patients with thromboembolic disease, it appears that each episode is accompanied by an increased tolerance to heparin. If no increase in tolerance to heparin occurs, it may be assumed that the patient will not develop thromboembolic disease. Thus, the tests may be used as a basis for the administration or the withholding of anticoagulant therapy. Admittedly, more cases will still be treated than will subsequently develop thromboembolic complications, but a substantial group of patients can be safely spared anti-coagulant therapy.

SUMMARY AND CONCLUSIONS

Methods are given for the measurement of heparin tolerance in the diagnosis of thromboembolic disease. Evidence for the consideration of these tests as indicators of the blood thromboplastin level is presented. Increased tolerance to heparin was found with great regularity in patients with thromboembolic disease.

It appears that excess circulating thromboplastin is the cause of the increased tolerance to heparin. The conclusion is drawn that a disturbance of thromboplastin is the basic etiology of thromboembolic disease.

We suggest that these tests be used as a basis for the employment of anti-coagulant therapy.

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CLINICOPATHOLOGIC CONFERENCE*

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CLINICAL FINDINGS

History. Mrs. M. Z., a 40 year old white, Polish woman, was admitted to the hospital, June 14, with the following complaints: lower abdominal pain and tenderness, protrusion of the cervix from the vagina on standing, frequent menstruation and partial urinary incontinence. Seven months prior to admission, the patient noticed that the cervix protruded from the vulva when she was standing. At this time she began to have pain in the lower part of the abdomen, vaginal discharge and dysuria. There was also leakage of urine from the bladder on coughing or straining.

The past history revealed that there had been an operative suspension of the uterus six years previously, and that the patient had had a large goiter since puberty. There had been two normal pregnancies, eight years and seventeen months ago. The patient had begun to menstruate at the age of 15. The periods had been regular, with intervals of twenty-eight days and normal flow for four or five days, until four months prior to admission when she began to menstruate every two weeks for three or four days. She had mild dyspnea on exertion. There was no history of gastro-intestinal disturbance. The patient's father had died of carcinoma of the lip; her mother was living and well.

Physical examination revealed an obese woman with marked oral sepsis with caries and pyorrhea. The thyroid gland was generally enlarged, irregular and nodular. The lungs were normal. There was no apparent enlargement of the heart; the rhythm was regular, the sounds were normal and there were no murmurs. The systolic blood pressure was 132 mm. of mercury and the diastolic pressure was 80 mm. The abdomen was obese, with a large incisional hernia. There was tenderness in the left hypochondrium and over the sigmoid colon. The pelvis showed marked relaxation, cystocele and rectocele and second degree descensus of the uterus. The cervix was large, eroded and bilaterally lacerated. There were extensive venous varicosities on both legs.

Laboratory data. The blood count revealed the following: hemoglobin 13.5 gm.; erythrocytes 4,380,000; leukocytes 6050; the differential count: polymorphonuclear leukocytes 51 per cent (filamented, 44 per cent; nonfilamented, 7 per cent); lymphocytes 42 per cent; monocytes 3 per cent; eosinophils 4 per cent. Urinalysis revealed: acid reaction, specific gravity 1.012, albumin and sugar negative. A Kline test was negative. Clotting time was three and one-half minutes.

Hospital course. On June 18, after preoperative medication of morphine sulfate grains $\frac{1}{4}$ and atropine sulfate grains $\frac{1}{150}$, an anterior and posterior colporrhaphy and Sturmdorf amputation of the cervix were done under gas induction-ether anesthesia. The patient was in the operating room one and one-half hours and returned to her room in good condition.

* Received for publication, August 12, 1947.

The immediate postoperative course was uneventful except for an elevation of temperature to 102.4 F. on the first day and a moderate degree of abdominal pain. Urinalysis on the day following the operation showed 1 plus albumin with 8 leukocytes and numerous erythrocytes per high power field; the urinary output through the indwelling catheter, inserted at the time of operation, was normal. The patient's temperature varied from normal to 101.4 F. during the second to fifth days postoperatively and her general condition was quite satisfactory. The catheter was removed on the sixth postoperative day; following this, the patient had some frequency and dysuria. On the tenth day following the operation, the patient complained of pain in the chest, but the temperature and pulse remained normal and examination of the lungs was negative. The chest pain subsided after a few hours but on the eleventh postoperative day, the patient experienced a sudden severe pain in the left knee and leg while walking to her bed from the bathroom. There was also marked pain in the lower abdomen at this time. Immediately following this, the patient developed the appearance of shock; the skin was cold and moist, there was marked pallor, and respirations were rapid and shallow. The heart was regular, the rate 108, and there was a loud, harsh systolic murmur heard over the entire precordial region with maximum intensity in the third intercostal space to the left of the sternum. The radial pulses were very small. The systolic blood pressure was 142 mm. of mercury and the diastolic was 82 mm. in the right arm. In the left arm, the systolic pressure was 142 mm. and the diastolic 100 mm. The abdomen was soft and not tender. The left leg and foot were cold and the skin showed a marked purplish red mottling. Arterial pulsations were absent in the left foot and there was apparent anesthesia. The clinical diagnosis at this time was mural thrombosis of left ventricle of heart with arterial embolism to the left leg.

The patient was put at absolute rest. The left leg was elevated and a heat cradle applied. A few hours later, the patient vomited three times, the vomitus consisting of undigested food. She recovered from the shocklike condition and did not complain of pain in the left leg until the following day when the temperature rose to 102.4 F., the pulse rate to 128 and the respiratory rate to 28. The following day the temperature rose to 103.2 F., the pulse became more rapid, the patient was semicomatose, and there was severe pain in the left extremity. The left leg and foot continued to show a marked grade of mottled purplish red discoloration and the foot was cold. The temperature and pulse rate rose progressively, the patient became comatose and developed jerking and twitching movements of the extremities and face, and died on July 2, the third day following the postoperative accident. At this time there was a well marked line of demarcation on the foot about 5 cm. above the toes with beginning gangrene below this point.

AUTOPSY REPORT

Gross findings. The important findings are summarized. The body was that of a well developed, well nourished white woman, measuring 170 cm. in length and weighing approximately 170 pounds. There was a midline suprapubic old

operative scar which appeared well healed except for a diastasis of the recti muscles. The pupils were round and equal and measured 4 mm. in diameter. There was no cyanosis, edema, or jaundice present, except for blotchy, purplish discoloration of the left leg and marked reddish purple discoloration of the toes and dorsum of the left foot for a distance of 5 cm. above the toes. At this point on the foot there was a sharp line of demarcation. No free fluid was found in the peritoneal cavity. There was a small amount of fresh blood in the posterior cul-de-sac. No free fluid was found in either pleural cavity. The visceral and parietal layers of the pleura over the left lung were adherent throughout by dense adhesions so that the substance of the lung was torn in removing it. The pericardial sac contained a normal amount of clear straw-colored fluid. The pulmonary artery was opened and no embolism was found on either side. There was no infarction, tuberculosis, or other type of consolidation in either lung. The bronchi were normal. The right pulmonary artery was normal and contained no thrombi or emboli. A branch of the left pulmonary artery contained a small antemortem thrombus. The heart weighed 440 gm. The pericardium and epicardium were normal. The muscle was light reddish brown and fine yellow streaks were present. The right atrium was dilated, grade 1. Extending from the region of the orifice of the superior vena cava to the crista terminalis there was a weblike network with long threads of attachment extending to the anterior margin of the fossa ovalis and to the interatrial septum 1.5 cm. above the ostium venosum. One strand also extended across to attach to the upper portion of the orifice of the inferior vena cava. There was no eustachian valve. The thebesian valve was well defined and about it there were no anomalies (Fig. 1). The fossa ovalis measured 2 x 2.5 cm. It was partly fenestrated and along its anterior margin in the region of the attachment of the reticulum there was a patency which measured 1.5 x 1 cm. In the meshes of the reticular network a layered, firmly attached thrombus was found which measured 1.5 x 1 x .5 cm. (Fig. 1). The right ventricle was hypertrophied, grade 2. The left atrium and ventricle were normal in appearance, but revealed dilatation, grade 1. The valves and endocardium were normal throughout. The coronary vessels revealed sclerosis, grade 1. Measurements of the heart: thickness of ventricular walls; left, 1.5 cm., right, 7 mm.; diameter of ventricles; left 8 cm., right, 9 cm.; circumference of valves; mitral, 8 cm., aortic, 6 cm., pulmonic, 6.5 cm., tricuspid, 11 cm. The septum was normal in appearance. The entire gastro-intestinal tract was free of lesions. The spleen weighed 560 gm. The surface was smooth and the color grayish purple. The splenic artery was completely thrombosed and the entire spleen was undergoing a grayish purple and hemorrhagic discoloration due to infarction. The splenic vein was occluded by an agonal type of thrombus. The pancreas was normal. The liver weighed 2075 gm. and was of normal consistency. The surface was reddish brown; the capsule was smooth and glistening. On cut surface it showed fairly good-sized areas of yellowish, lusterless mottling. There were multiple adhesions about the gallbladder which was filled with small stones. The wall of the gallbladder was thickened. The



FIG. 1. Right atrium which has been opened and reveals the thrombus attached to the network at A. The orifice and valve of the coronary sinus are seen in the lower left just above the tricuspid valve; just above this the orifice of the inferior vena cava, and at the top above the network the orifice of the superior vena cava. The fossa ovalis is clearly seen in the lower left center and its patency is identified by a probe which has been passed from the left to the right atrium.

common duct was dilated, grade 2, and on exploration five small stones were found imbedded in its duodenal end, apparently causing an incomplete obstruction. The suprarenal glands were normal. The right kidney weighed 186 gm., the left, 210 gm. The capsule stripped easily leaving a rough surface, the roughening being due to large scars and depressed areas. Fetal lobulations were prominent. The color was a grayish pink, varying to mottled dark red. The cut surface was dark red in color with the exception of the pyramids which were blanched. The normal tubular and glomerular markings were obscured. The right renal artery was thrombosed in its main portion and branches of the left renal artery were thrombosed. The pelves, calices and ureters were normal on both sides. The urinary bladder was normal. The cervix was hemorrhagically discolored and the superficial epithelium was partly necrotic. The main portion of the cervix was recently amputated. The uterus was small, appeared normal and measured 6 x 4 x 3.5 cm. The uterine and adnexal veins were not thrombosed. The tubes and ovaries were normal. The aorta revealed sclerosis, grade 1. It was examined as far as the femoral artery on each side and no thrombi were found. The vena cava, iliac and femoral veins revealed no thrombi. The thyroid was nodular and enlarged. The brain was not examined.

Microscopic findings. The suprarenal glands revealed only congestion. Four sections of kidney revealed large areas of circumscribed coagulation necrosis with polymorphonuclear neutrophilic leukocytic infiltration and hemorrhage, especially at margins. There were multiple areas of central and focal necrosis in the central and intermediate lobular positions of the liver which were infiltrated by polymorphonuclear leukocytes. There were large numbers of lymphocytes in periportal spaces. Sections of the spleen revealed thrombosis of branches of the splenic artery within the spleen. The splenic tissue was undergoing circumscribed coagulation necrosis and in the necrotic zones there was evidence of hemorrhage and edema. Sections of the lung revealed extensive extravasations of serum into the alveoli. In certain areas there were a few polymorphonuclear leukocytes present within the fluid. The vessels were congested and a few of the pulmonary arteries contained small embolic masses of layered antemortem blood clot. The thrombus from the network of the right atrium was of recent formation, layered type, free from organization and infection. There was no significant change in the myocardium except in one section of the left ventricle which revealed an area of myocardial atrophy and dense scar tissue replacement. Sections stained for lipoids by sudan IV revealed a severe grade of lipid degeneration of the myocardium. Atrophy of glandular tissue with adipose tissue replacement were the only changes found in the pancreas. In the uterus the endometrium was hypertrophied and in the proliferative phase of the cycle. The glands were hypertrophied and dilated. There was no evidence of infection.

■ The anatomic and microscopic diagnoses were as follows: recent (15 day) anterior and posterior colporrhaphy, Sturmdorf amputation of cervix and peri-

neorrhaphy; reticular network in right atrium (remnant of left venous valve and septum spurium); patent foramen ovale; primary thrombosis in network of right atrium with residual thrombus in network. Paradoxical embolism of splenic artery, renal arteries, left tibial artery (clinical), and cerebral arteries (clinical) with gangrene of left foot and infarction of kidneys, spleen and brain (clinical); fatty degeneration of myocardium; pulmonary embolism (small), no pulmonary infarcts; edema of lungs with beginning bronchopneumonia; focal and central necrosis of liver; splenomegaly (560 gm.); thrombosis of splenic vein (agonal); hypertrophic persistent proliferative endometrium (hyperestrinism); cholelithiasis and choledocholithiasis; chronic fibrous pleural adhesions (left); small scar of myocardium (septum); adenomatous goiter; atherosclerosis of aorta, grade 1.

DISCUSSION

This case is remarkable because it presents an unusual combination of congenital cardiac anomalies, namely, reticular network in the right atrium, the abnormal remains of the left venous valve and septum spurium and patent foramen ovale. Of additional interest is the relationship of these anomalies to the fatal postoperative complications of thrombosis, paradoxical embolism, infarction and gangrene. The cardiac anomalies were probably responsible for the unfavorable outcome in a patient who otherwise probably would have recovered. The surgical procedures were simple and locally without serious complications. Congenital defects of the heart are relatively rare, comprising about 2 per cent of the total number of cases of organic heart disease and about 5 per cent of the cases in children. About one-half of these congenital anomalies are not clinically important in themselves but are likely to become the seat of serious infections or predisposing causes to cardiac disease.

It is estimated that almost 25 per cent of normal adults have a patent foramen ovale. The opening is usually small, oblique or valvular in character, allowing the passage of very little blood from one side of the heart to the other. There are usually no clinical evidences of this type of patency. At times the opening is larger, and through it a thrombus, reaching the right atrium from one of the large veins, may pass to the left side of the heart and cause embolism of the brain, or some organ other than the lung: so-called crossed or paradoxical embolism. This type of patent foramen ovale usually causes no other trouble. There are no physical signs. Larger patencies of the foramen ovale constitute one of the clinical types of cardiac anomalies of the "cyanose tardive" variety.

Anomalies of the valves of the right atrium may be present, and are proportional to the degree of regression of the embryonic valves, namely, the right and left valves of the sinus venosus, the septum spurium and sinus septum. When there is defective development of the interatrial septum there may be various grades of persistence of any of these valvular structures. The position of these valves in the adult human heart is as follows. Rudiments of the right venous valve are found along the anterior rim of the orifice of the inferior vena

cava extending upward to the upper portion of the crista terminalis and downward across the orifice of the coronary sinus toward the tricuspid valve. Remnants of the left venous valve are found on the interatrial septum in the region of the rim of the fossa ovalis. Remnants of the septum spurium are found anterior to the mouth of the superior vena cava near the interatrial septum. Remnants of the sinus septum extend from the rim of the fossa ovalis to the orifice of the inferior vena cava in the region of the eustachian valve.

The most frequently described persistence of valves of the right atrium is that reported by Chiari² in 1897 and known as Chiari's network. This consists of a network of fine or coarse fibers in the right atrium extending from the interatrial septum on the upper portion of the crista terminalis to the thebesian or eustachian valve or to the region of the orifice of the coronary sinus and the inferior vena cava. Chiari considered the network he described as due to persistence, in the form of a reticulum or network, of remains of the septum spurium and the right venous (or eustachian) valve. He described eleven cases. In one of the cases, the network was responsible for the death of the patient. The patient was a man, age 24, who had extensive pulmonary embolism, the source of which was apparently a thrombus lodged in the fibers of the network. Although the term "Chiari's network" is sometimes used loosely for any of the networks or reticula which occur in the right atrium resulting from remnants of various valves, its proper usage should be restricted to the networks when they are remains of the eustachian valve.

Anomalous remains of the various valves have been recorded in the medical literature a number of times. A complete review of the subject may be found in the exhaustive article by Yater,⁵ in which twenty-two cases of typical Chiari's network from the literature are recorded. He described eleven additional cases of abnormal reticula in the right atrium, four of which were of the Chiari variety. The diagnosis was made by postmortem examination in all cases. Yater estimated that this anomaly occurs in 2 to 3 per cent of hearts which are otherwise normally developed. Helwig³ stated that it is found in 1.5 per cent of routine autopsy examinations. These reticular formations are usually not of clinical significance and produce no symptoms nor signs. Alvarez and Herrmann¹ reported a case in which they thought a peculiar cardiac murmur might have been due to a Chiari network. Wilson⁴ recently reported a case of Chiari's network associated with a murmur resembling the bruit de Roger, and suggested that this type of murmur might be a sign of this particular anomaly.

The greatest significance of this condition is that it affords, in a certain number of cases, a site for the formation of thrombi on the threads of the network. This may result in pulmonary embolism. Yater states that, paradoxically, such a network may prevent the occurrence of pulmonary embolism by ensnaring an embolism from a vein.

It is difficult to ascertain accurately in chronological order, all of the events which preceded the fatal embolic accidents experienced by this patient. Following operation an unexplained fever developed. Possible sources of infection

included the stump of the amputated cervix uteri and the urinary bladder which contained the indwelling catheter. In the course of this, myocardial lipid degeneration and central necrosis of the liver supervened. Thrombosis was thus favored and probably occurred on the web and threadlike reticulum of the right atrium. Thrombi from this source were detached, a few entering the pulmonary arteries and others passing through the patent foramen ovale to the arterial circulation, from which the various embolic accidents developed.

The gynecologic conditions for which the patient came to surgery were (1) metrorrhagia, related to a hyperestrin state and persistent proliferative endometrium; and (2) pathologic changes of the cervix uteri related to procidentia. The previous uterine suspension altered the surgical procedure of choice, which probably would otherwise have been vaginal hysterectomy and colporrhaphy. The continued existence of procidentia, as in this case, is rather frequent and indicates the judgment which is necessary, in selecting the operation for relief of this common gynecologic condition, so that recurrence will not result.

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EDITORIALS

CARDIOLIPIN LECITHIN ANTIGEN

Cardiolipin lecithin antigen described by Pangborn^{18,18a} in 1941, is a brilliant new development in the serodiagnosis of syphilis.

The story of the search for the active antigenic factor in tissue extracts starts with the report of Marie and Levaditi,¹⁴ in February 1907, ten months after the introduction of the Wassermann test.²⁵ These investigators found that syphilitic spinal fluid gave positive Wassermann reactions not only with the carbolized saline extract of a syphilitic liver and spleen, as specified, but also with a similar extract of normal human liver. Several months later, in May, Weil²⁷ reported positive reactions with mixtures of syphilitic serum and carbolized saline extract of sarcomatous tissues. On the basis of his observations and those of Marie and Levaditi, Weil questioned the specificity of the Wassermann test. He thought that the reaction was due to some protein or other tissue element and not to spirochetal substance.

Weil's challenge led to intensive studies of the nature of the "antigen" for the Wassermann test and reports relating to it quickly appeared in the literature. Weygandt²⁹ and Plaut,²¹ in July, Michaelis¹⁵ and Fleischmann,⁶ in September, and Braun and Weil,²⁵ on December 9 of the same year, reported the presence of an antigenic fraction in extracts of normal organs and considered it to be a special protein substance. On December 12, Landsteiner, Müller and Pötzl¹¹ published their revolutionary discovery that the active tissue factor was alcohol-soluble and apparently a lipid. On the sixteenth of the same month, Wassermann²⁶ reported that his associates, Porges and Meier, had found an active antigenic fraction in syphilitic and in normal tissues that was alcohol-soluble and that pure lecithin also gave positive reactions with syphilitic serum. On December 21, Levaditi and Yamanouchi¹² corroborated the findings of Landsteiner and associates and of Porges and Meier. The latter investigators reported their findings in full in April 1908.²² As a result of these fundamentally important findings, stable alcoholic extracts of nonsyphilitic liver or heart (human or animal) were routinely used as antigen in the Wassermann test and quickly replaced the carbolized saline extracts of syphilitic tissues which, ordinarily, could be used satisfactorily for five or six days only.

After the discovery that the active antigenic fraction was a lipid and similar to lecithin, Noguchi and Bronfenbrenner,¹⁷ in 1911, found that the acetone insoluble fraction of ether and alcohol extracts of animal and human livers and of animal hearts gave much more specific results in the complement-fixation test for syphilis than did the crude alcoholic extracts. They found that the acetone-soluble portion contained fats, fatty acids, soaps, cholesterin and other substances, possessed hemolytic and anticomplementary properties and, occasionally, caused false positive Wassermann reactions.

Continuing the search for the active factor, Neymann and Gager,¹⁶ in 1917, reported a study of the antigenic properties of the various phosphatides extract-

able from beef heart powder by the method of Erlandsen.⁴ According to the latter, ox heart muscle contains the following phosphatides: (1) monoamido-monophosphatide (lecithin-cephalin group; N:P = 1:1), (2) monoamido-diphosphatide (euorin; N:P = 1:2), (3) diamido-monophosphatide (secondary lecithin; N:P = 2:1) and (4) diamido-diphosphatide (jecorin, protagon; N:P = 2:2). They found that the diamido-monophosphatide fraction (in the secondary alcoholic extract) was by far the most potent antigenic lipid present, binding complement in a titer of 1:12,800 whereas the next best antigenic lipid, the lecithin fraction of the primary ether extract, bound complement only to a titer of 1:800. This report influenced the serodiagnosis of syphilis for the next thirty years. It was responsible for the use of antigens prepared by alcohol extraction of beef heart powder previously extracted with ether and for the belief that diamido-monophosphatide was the most active antigenic lipid present. These antigens and others containing the acetone-insoluble fraction of beef heart extracts were employed in the standard tests in this country. Although they gave much better results in the serodiagnosis of syphilis than did plain or cholesterinized alcoholic extracts, they are found in the American Serological Conferences of 1934⁵ and of 1941²⁴ to give a large percentage of nonspecific results in leprosy (37.8 to 72.5 per cent) and in malaria (8 to 19 per cent) and occasional nonspecific reactions in other conditions and in normal individuals. Although the results were good with the standard antigens for syphilis, it was clear to serologists that improvement was possible.

The apparent end of the search for the active antigenic factor in tissue extracts that combines with syphilitic reagin came with the masterful work of Pangborn, first reported in 1941.^{18,15a} Employing a method requiring many steps, Pangborn isolated from beef heart muscle, in essentially chemically pure form, a previously unknown non-nitrogenous phospholipid and named it cardiolipin. In addition, from the same source, by rigorous methods of purification, she obtained lecithin free of impurities. Pangborn found that although cardiolipin alone and purified lecithin alone were each serologically inactive, certain mixtures of the two acted well as an antigen in a complement-fixation test for syphilis. Subsequently, Pangborn¹⁹ offered the following criteria for standardizing cardiolipin and lecithin:

LECITHIN

1. Gelatinous appearance in anhydrous ether,
2. Concentrated ethereal solution completely colorless,
3. Contains not more than traces of alcohol insoluble material,
4. Contains not more than 0.05 per cent nitrogen,
5. Phosphorus content 4.18 per cent,
6. Iodine number 115 or higher.

LECITHIN

1. Concentrated solution colorless,
2. NP ratio, 1N: 1.12 P,

3. Phosphorus content, 3.95 per cent,
4. Contains less than 0.05 per cent amino nitrogen,
5. Iodine number 75 to 85.

Recently Pangborn²⁰ reported the calculated formula of the sodium salt of cardiolipin as $C_{150}H_{265}O_{24}P_3Na_3$ (mol. wt. 2195) and the iodine number as 120-125.

It now seems probable that the various phosphatides studied by Neymann and Gager were not chemically pure and that the antigenic potency of the different lipoids they isolated depended upon the quantity of cardiolipin and of lecithin present.

Although cardiolipin lecithin antigen has been studied by serologists in complement-fixation and flocculation tests for syphilis (including Kolmer, Kahn and Kline tests) for the past six years only, all the reports^{1, 2, 3, 7, 8, 9, 10, 13, 23} have been favorable, attesting to its purity, reproducibility in lot after lot, and to its stability. It has been found that the different tests require different quantities of cardiolipin and of lecithin for optimal results.

The following three communications clearly indicate the superiority of cardiolipin lecithin antigen over present day standard antigens: Rein and Bossak,²³ in January 1946, reported that cardiolipin lecithin antigen in the slide test gave results of much greater specificity in cases of malaria than did the Kline antigen in the diagnostic and exclusion slide tests and the Mazzini antigen in the Mazzini test. Furthermore, the slide test with cardiolipin lecithin antigen gave results of greater sensitivity in syphilitic cases than did the Mazzini, Kahn, Kolmer and Kline diagnostic tests.

In a report in February 1946,⁹ only 1 per cent nonspecific results was obtained with optimal cardiolipin lecithin antigen in the Kline slide test on 101 serums of patients with malaria as against 8 to 24 times as many false positive reactions with one or other of the standard Eagle, Hinton, Kahn, Kline or Mazzini antigens in the same test. Furthermore optimal cardiolipin lecithin antigen in the slide test, gave more sensitive results in syphilitic cases than did Hinton, Kahn or Kline antigens. In addition, optimal cardiolipin lecithin antigen gave more sensitive results in the spinal fluid test than did the most sensitive test with Kline antigen.

In a subsequent communication,¹⁰ it was reported that whereas the diagnostic slide test with Kline antigen in 8862 tests of general hospital and ambulatory patients gave 0.3 per cent false positive reactions, the test with optimal cardiolipin lecithin antigen gave only 0.06 per cent nonspecific reactions.

It should be mentioned that, like the standard antigens, cardiolipin lecithin antigen is not altogether specific in that it also gives many false positive reactions in cases of leprosy²³ and with serums of many lower animals.⁹

Notwithstanding the fact that chemically pure cardiolipin lecithin antigen is not completely specific for syphilitic reagin, the evidence already at hand indicates that it is far superior to all present day standard antigen extracts and should soon replace them. Furthermore, cardiolipin lecithin antigen, the best reagent for the detection of syphilis now known, may serve well as a base for a single standard blood test for the disease. It is not too much to say that Pang-

born's discovery is one of the most important contributions to the serodiagnosis of syphilis since the introduction of the Wassermann test.

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ACCURACY OF CHEMICAL ANALYSIS IN CLINICAL LABORATORIES

The Medical Society of the State of Pennsylvania and the Pennsylvania Association of Clinical Pathologists are to be congratulated on undertaking surveys to check the accuracy of chemical analyses in clinical laboratories in their state.

The survey covered quantitative tests for glucose, sodium chloride, urea, calcium, total protein, albumin, globulin and hemoglobin. Most of these tests may be considered to be among the simpler types of biochemical tests and, while it was to be expected that some variation in results would occur, the degree of disparity of results was astonishing. This, however, has also been the usual experience in surveys of laboratories for accuracy of serologic or bacteriologic tests. Such surveys, when periodically repeated, have served the excellent purpose of increasing the accuracy of results.

Some years ago, the American Society of Clinical Pathologists took the initiative in fostering, on a national basis, evaluation studies of serologic tests for syphilis which resulted in clarification of the problems involved in the use of such tests and in the adoption of recommendations for procedure and interpretation. At various times, the Society has also assigned to committees of experts chosen from among its members, the study of similar problems in neoplasia, hormonology, hematology, chemistry, bacteriology and mycology. The Society may thus render valuable service in formulating or helping to formulate recommendations for terminology, general rules of procedure, techniques to be used and methods of interpretation. The conduct of surveys is best assumed by State Pathologic Societies either alone or in cooperation with State Medical Societies. In the Pennsylvania survey it is encouraging to see how well the cooperating laboratories voluntarily participated; for there was no compulsion or legal pressure such as might be exerted by State Boards of Health conducting similar surveys. It is to be hoped that the Pennsylvania survey will be periodically repeated and that surveys will also be made of other branches of clinical pathology. It is to be hoped, too, that other State Pathological Societies, or groups of State Pathological Societies in sparsely populated regions, will follow the example of Pennsylvania.

The effect of such periodic surveys and of formation of committees on standardization is bound to mean a continuing increase in accuracy of clinical laboratory work, which permits increased accuracy in clinical diagnosis, which in turn promotes the increased health of the patient.

SELECTED ABSTRACTS

Culture Experiments on the Intestinal Flagellates. I. Trichomonads and Other Flagellates Obtained from Man and Certain Rodents. D. H. WENRICH. J. Parasitol., 32: 40-53, 1946.

In growing flagellates the custom has been to make transplants at frequent intervals because of the belief that the formation of noxious waste products plus the exhaustion of food supplies hindered their growth. Wenrich, instead of making transplants, adds water and nutrients to the tubes and finds the organisms grow better and survive for much longer periods. His culture medium consists of Drbohlav's modification of Ringer's fluid (NaCl 6.0 gm., KCl 0.1 gm., CaCl_2 0.1 gm., NaHCO_3 0.1 gm., water 1000 ml.) or ordinary Ringer's solution with 0.8 per cent NaCl to which is added sufficient Loeffler's dried blood serum (Difco) and dried gastric mucin (Ratliff, H. L., Proc. Soc. Exper. Biol. and Med., 31: 602, 1933-34) to make 0.2 per cent each. Where water is needed these ingredients are dissolved in it; otherwise they are added as powder.

Rochester, New York

W. S. THOMAS

Physiology of Bacteria-Free Trichomonas vaginalis. J. GARTH JOHNSON. J. Parasitol., 33: 189-200, 1947.

This paper is a running commentary on seven years' experience in the development of a more precise culture medium for this organism. The author arrived at a medium sufficiently optimum that one or two organisms seeded in 10 ml. of culture fluid provided several million in a limited period of incubation. His medium contains the following ingredients: cystine, peptone, liver infusion, maltose, human blood serum, modified Ringer's solution (0.6 per cent NaCl) and a small amount (0.1 per cent) of agar. The optimum temperature was found to be from 35 to 39 C., the optimum pH from 5.5 to 6.0. and the maximum growth occurred in an oxygen-free medium.

W. S. THOMAS

Further Experimental Studies on the Role of the Plasma Cells as Antibody Producers. M. BJORNEBOE, H. GORMSEN AND F. LUNDQVIST. J. Immunol., 55: 121-129, 1947.

The senior authors, in previous communications, have reported evidence which they believe indicates that plasma cells are a source of antibodies. This report covers further investigation.

It had been noted previously that in the fatty tissue of the renal sinus of highly immunized rabbits, there were large infiltrations of cells which were almost entirely plasma cells. In the present experiments rabbits were immunized for one to eight months with large doses of pneumococcic vaccine. Tissues were removed, frozen and ground and examined for antibody content. Fatty tissue from the renal sinus containing large amounts of plasma cells was compared with fatty tissue from retroperitoneal fat and thymus. The amount of antibody in the adipose tissue from the renal sinus which was filled with plasma cells was much greater than was found in the other sources.

These findings are interesting in that the current belief is that antibodies arise chiefly from lymphocytes.

Dallas, Texas

J. H. BLACK

A Fatal Hemolytic Reaction Following Transurethral Resection of the Prostate Gland. C. D. CREEVEY AND E. A. WEBB. Surgery, 21: 56-66, 1947.

Hemoglobinemia, jaundice and progressive renal failure were noted in a patient following transurethral resection of the prostate. At autopsy, hemoglobin casts were found in convoluted and collecting tubules. This and similar observations of hemolysis following such operations is explained by the authors by means of the following attractive hypothesis. During this type of operation, the field in the urinary bladder is kept clear and free of blood by irrigation with large amounts of sterile water. Saline cannot be used because it is a

good conductor of electricity and would interfere with electrocautery. The water laves the blood and the considerable amount of pressure may, according to the authors, force both water and hemolyzed blood into the prostatic veins and then into the general circulation. The final effect would be the same as a transfusion of incompatible blood: a hemolytic transfusion reaction with manifestations indistinguishable from those in the patient reported here. The effect may be aggravated by other factors known to cause postoperative uremia: hemorrhage and hypotension due to loss of blood, both leading to vasoconstriction and pyelonephritis.

The hypothesis sounds plausible and should be checked by those who have access to suitable urologic material. All that is necessary is to obtain a specimen of blood from such patients within a few hours after operation, employing the usual precautions for prevention of hemolysis. Hemoglobinemia would corroborate the thesis of this paper. Icterus index done twenty-four and forty-eight hours after operation may show a rise. Later, but only in the most severe cases, renal failure may be observed with progressive oliguria and progressive azotemia.

The authors warn that such serious complications occur only in cases when an attempt is made to remove the gland to the capsule.

Chicago

I. DAVIDSOHN

Further Observation on Post-Tracheotomy, Mediastinal Emphysema and Pneumothorax. G.

B. FORBES, G. SALMON AND J. C. HERWEG. *J. Pediat.*, **31**: 172-194, 1947.

Mediastinal emphysema (25 per cent) and pneumothorax (10 per cent) complicating the post-tracheotomy state have engaged the interest of Forbes and his coworkers for several years. They suggest, as have others before them, that the emphysema is the result of forced inspiration in the presence of an inadequate airway and an open neck wound (tracheotomy). In their series, tracheotomy was performed for a variety of conditions. The pneumothorax is produced by the rupture of the mediastinal pleura by the overdistention of the mediastinum with air. Experimental studies on dogs, aided by the use of lipoidol and trypan blue as tracers, have confirmed their ideas about the genesis of emphysema and pneumothorax.

To overcome the inadequate airway, some cases were provided with an indwelling bronchoscope or intubation tube left in place during tracheotomy. There was 70 per cent greater incidence of mediastinal emphysema in the control group, and 8 times as much pneumothorax. Diagnostic x-ray studies were done in many instances to rule out pre-operative spontaneous emphysema and pneumothorax.

Fort Wayne, Indiana

S. M. RABSON

Role of Hemoconcentration in Production of Gastric and Duodenal Ulcer Following Experimental Burns. S. R. FRIESEN AND O. H. WANGENSTEEN. *Proc. Soc. Exper. Biol. and Med.*, **64**: 81-85, 1947.

Recent studies (Kapsinow, R.: *South. M. J.*, **27**: 500, 1934) on the production of gastroduodenal ulcer have suggested hemoconcentration as a responsible agent. Others (Hartman, F. W.: *Gastroenterol.*, **6**: 130, 1946) have discussed the rôle of sepsis. In the present instance, Friesen and Wangenstein immersed healthy barbiturate-anesthetized dogs in water at 100 C. for ten to fifteen seconds to produce 40 per cent, third degree burns. Another series of animals received, in addition, histamine-in-beeswax injections shortly before immersion. A third group was distinguished by the supplementary prevention of hemoconcentration by intravenous infusions of plasma (with and without glucose, saline, etc.).

The authors conclude that hemoconcentration is an important factor in the etiology of ulcer after burns. Histamine administration increased the incidence of ulcer. Prevention of hemoconcentration by proper therapy was an ulcer-prophylactic, even in those cases where histamine had been employed. Gastro-intestinal congestion, erosion and ulceration in burns, then, may be indices of the adequacy of therapy.

S. M. RABSON

Histopathology of the Gastric Semisquamous Epithelial Layer. FREDERIC DURAN-JORDA. Surg., Gynec. and Obst., 84: 983-989, 1947.

Duran-Jorda demonstrates the histopathology of gastro-intestinal erosions and ulcers with reference to the semisquamous epithelial layer. The existence of such a layer has been well substantiated by the author in an article published in *The British Journal of Surgery*, 33: 346-352, April 1946, entitled "A Micro-Incineration Study of the Flat Epithelial Layer Covering the Alimentary Tract". He describes the technic of formalin vapor fixation of fresh specimens and the micro-incineration of the tissue to prove that this layer is not an artefact or mucous debris. Through serial sections, alternately employing hematoxylin and eosin stains, micro-incineration and mucin stains, Dr. Duran-Jorda proves the existence of such a layer covering the inner surface of the entire gastro-intestinal tract, vermiform appendix and gallbladder.

Using fresh surgical specimens and formalin vapor fixation, a study of erosions, preulcerative lesions and ulcerations showed the semisquamous epithelial layer to be involved before any histologic changes occurred in the mucous membrane. The lesion in the semisquamous epithelial layer is an infiltration of round cells and polymorphonuclear leukocytes from the semisquamous layer. Cellular bridges extend to and between the mucous glands.

In gastric ulcers the semisquamous epithelial layer is missing except at the crater's edge. Healing of the ulcer begins with growth of epithelium from the edge and finally bridges the ulcer. The mucous membrane below projects finger-like groups of cells to form new glands and fill in the gap. The differentiation of preulcerative and postulcerative lesions depends on the absence or presence of a healthy semisquamous epithelial layer. In the latter this layer is present but without cellular bridges linking the mucous membrane.

A thorough study of this layer in relation to gastro-intestinal carcinoma has not been completed, but preliminary studies suggest that the mucous membrane is first involved by neoplastic changes. Erosion through the semisquamous epithelial layer occurs much later. The abstracter suggests that this may prove a valuable adjunct in determining whether the carcinoma arises in a peptic ulcer or in the absence of previous ulceration.

Detroit

O. A. BRINES

Epidemic Diarrhea of the Newborn in Massachusetts. A. D. RUBENSTEIN AND G. E. FOLEY. New England J. Med., 236: 87-94, 1947.

The etiology of epidemic diarrhea of the newborn has not been determined. Shigellae, salmonellae, *A. aerogenes*, *E. coli*, protei, alpha and gamma streptococci, *Ps. aeruginosa*, moniliae and filtrable agents have been blamed. The authors analyse 258 cases of such diarrhea as reported and investigated in Massachusetts during the last ten years.

Nineteen outbreaks, causing 85 deaths (33 per cent), were studied. Premature infants showed a higher mortality. Overcrowding, insufficient personnel, general use of a common rectal thermometer and inadequate supervision of formula-making were frequently encountered. Laboratory examinations of so-called "sterilizing solutions", including alcohol, thermometer dips, formulas and utensils showed heavy bacterial contamination. The ineffectiveness of disinfecting solutions, as alcohol and bichloride of mercury, was illustrated by experiments demonstrating that with the addition of small amounts of protein these solutions actually support bacterial growth.

In several outbreaks apparently identical, *Staph. aureus*, D group and A group streptococci were isolated. Group D streptococci were frequently encountered in the throats of infants with epidemic diarrhea. This suggests that, as a result of poor nursery technics, infants may be exposed to organisms normally present only in the intestines.

While the authors avoid drawing conclusions as to the etiologic factor of epidemic diarrhea of the newborn, this paper is a valuable study of nursery sanitation and throws much light on methods of spreading such infections.

San Juan, Puerto Rico

O. FELSENFELD

Comparative Distribution and Possible Pathogenicity of Paracolon Bacteria in an Area Highly Endemic for Enteric Infections. W. B. CHRISTENSEN. J. Bact., 53: 317-324, 1947.

In this paper, the nomenclature for paracolon organisms as proposed by Borman *et al.* is being used. The investigations were carried out in North Africa. Hospitalized patients, American troops and Arab food handlers were examined. Of the stools from patients suffering from diarrhea, 81.1 per cent harbored paracolon organisms, while only 47 per cent of persons without abdominal disturbance had stools containing these microbes. Paracolon bacteria were classified according to the scheme of Stuart *et al.* One type, *Paracolon aerogenes*, which did not form hydrogen sulfide, produced urease, fermented gelatin and salicin slowly, sucrose rapidly, was mostly motile, showed a typical IMViC reaction, was most frequently encountered in diarrheic stools from which salmonellae or shigellae could not be isolated. Most of these strains were serologically identical. The author believes that this type is pathogenic in the area which he studied and he joins Parr, Weil, Stuart *et al.*, Neter, Young and others in considering paracolon organisms as possible pathogens.

This paper is an excellent contribution to the discussion of organisms of doubtful pathogenicity. While it is based on a relatively small series of observations, great care was taken in the serologic and biochemical identification of the microbes.

O. FELSENFELD

Comparison of the β -Glucuronidase Activity of Normal, Tumor, and Lymph Node Tissues of Surgical Patients. W. H. FISHMAN AND A. J. ANLYAN. Science, 106: 66-67, 1947.

Determinations of the activity of β -glucuronidase were performed in malignant tumors and in their normal matrix. Significant elevations of the enzymic activity were noted in carcinomas and sarcoma of the breast and in some carcinomas of the gastro-intestinal tract. Also, lymph nodes with metastatic involvement contained higher amounts of the enzyme than uninvolved nodes of the same patient. Benign tumors showed no increase of the enzyme.

The material presented is small, fourteen primary and three recurrent malignant tumors, four metastases to lymph nodes, but the fact that all but three of the primary neoplasms exhibited the enzymic alterations makes it likely that a new biochemical characteristic of malignant tumors has been demonstrated.

β -glucuronidase catalyzes the conjugation of glucuronides, among which estrogen glucuronides play an important part. Hence further investigations will be required to elucidate the significance of this enzymic deviation in neoplasms and its possible relationship to hormonal factors.

Chicago

KURT STERN

Inactivation of the Virus of Infectious Hepatitis in Drinking Water. J. R. NEEFE, J. B. BATY, J. G. REINHOLD AND J. STOKES, JR. Am. J. Pub. Health, 37: 365-372, 1947.

Coagulation, setting and filtration of contaminated water did not eliminate or inactivate the hepatitis virus, as the disease developed in 40 per cent of volunteers who ingested such treated water. Neither was the contaminated water disinfected to provide, by sufficient chlorine, after thirty minutes of contact, a concentration of 1.1 p.p.m. total and 0.4 p.p.m. free residual chlorine. However, thirty minutes of chlorination of the contaminated water pretreated by coagulation, setting and filtration did inactivate the virus.

Demonstration of Hormesis (Increase in Fatality Rate) by Penicillin. W. A. RANDALL AND H. WELCH. Am. J. Pub. Health, 37: 421-425, 1947.

It was observed that in white mice infected with *E. typhosa*, penicillin in certain doses exerted a hormetic (stimulating) influence on the fatality rate, which was not due to impurities in the drug, but seemed to be caused by an *in vivo* stimulation in growth of the infecting organism.

BOOK REVIEWS

Excerpta Medica. Section XII, Ophthalmology, Section XIV, Radiology. Amsterdam, The Netherlands: Excerpta Medica Inc., 1947.

Excerpta Medica Incorporated of Amsterdam is preparing a series of abstract journals covering the whole field of medical science. The abstracts are written in English and are edited in the Netherlands. The chief editors are Professors M. W. Woerdeman, anatomist, A. P. H. A. De Kleyn, otolaryngologist, and W. P. C. Zeeman, ophthalmologist, all of the University of Amsterdam. Each of the fifteen sections has separate editors, experts in their fields from various countries, including the United States. They will be assisted by a staff of technical assistants and linguists.

According to the prefaces of the sections the first objective is completeness. The principle adopted is to prepare for publication in each section every article on the subject appearing in the medical literature of the world. The second objective is to prepare informative abstracts. To insure this the work of abstracting is placed in the hands of competent specialists, and all published abstracts will bear the signature of the abstracters. In the section on Radiology no mention is made of books, but the section on Ophthalmology contains a summary of a Russian monograph, and presumably at least some book or monograph material will be abstracted. The third objective is to publish the abstract as soon as possible after the article is published, but in the issues which will make up the first volume of each section there may be articles dating back as far as 1940. The editors state that the reason for this is the inaccessibility of the articles appearing during the years 1940-45 and the importance of some of the work published during this period.

Of 146 abstracts in the section on Radiology, 65 are from American journals, 49 from British, 17 from Scandinavian, 9 from Swiss and only 6 items from all other sources. No abstracts from German literature are included. This omission is serious for American users, and since the index is in English it is no doubt expected that most subscribers will be English-speaking. Presumably, this omission will be corrected when the German literature becomes more readily available.

The length of the abstracts varies considerably from one line to well over a page. The abstracts are collected under certain headings which, of course, will make it easier for the specialist to find the material in which he is most interested. An author list appears in each number. Each journal is to be issued monthly, is to have one or two volumes a year depending on the amount of material, and each volume is to have an index.

If the journal can take the place of the German Zentralblätter and Berichte as it hopes to do, the venture will be very worth while. If it cannot, it will no doubt die a natural death, and the field of clinical medicine will still need an abstract service.

Medical Science Department, Public Library
Detroit

MARJORIE J. DARRACH

Die Milzpunktion. Technik, klinisch-diagnostische und hämatologische Ergebnisse. By SVEN MOESCHLIN, Privat-dozent an der medizinischen Fakultät der Universität Zürich. 205 pp., 55 figs. 32 tables. Bound, 30 francs. Basel: Benno Schwabe and Company, 1947.

This monograph deals with the technic, diagnostic and hematologic aspects of splenic puncture. It is based upon 180 punctures which are critically compared with those of other authors. The author states that he advocates splenic puncture as an adjunct to, but not as a replacement for sternal marrow and lymph node puncture.

In the introduction, he explains that this method, with proper technic and indication, is entirely free from danger providing the following conditions are adhered to:

1. The spleen must be definitely enlarged.
2. It is contraindicated in cases of hemorrhagic diatheses.
3. Splenic puncture should not be undertaken in a freshly septic, or in a painful spleen.
4. Numbed patients should not be punctured.
5. It should be done only under rigid aseptic conditions.

The puncture should be done in the region of the inner half of the ninth or tenth intercostal space a little more lateral or more medial, during deep inspiration, according to the size of the splenic outline.

He utilizes a needle from 12 to 15 cm. long with a diameter of 1.2 to 2 mm. with a cannula and a rider, and a tight fitting 20 cc. Record syringe. Where a specific needle is not attainable, a lumbar puncture needle may be used.

The material must be quickly blown out of the needle as it clots even more quickly than sternal marrow. Smears are stained with Giemsa's method and particles of tissue embedded in paraffin.

After the puncture, the patient should remain flat on the back for one hour, then in bed for six hours longer. In 180 punctures, there was no instance of hemorrhage. The only symptom was a complaint by some patients of a slight pain in the left shoulder region on respiration lasting from one-half to one hour. In only three patients did it last for two to three days. This was controlled with a dilaudid suppository. The puncture itself is painless, providing the anesthesia is properly carried out.

Then follows a discussion of the types of cells found in splenic punctures with a description of each cell, beautifully illustrated by photomicrographs and water color, in various types of splenomegaly, in infections including infectious hepatitis and brucellosis, as well as in the leukemias and reticuloses.

The author divides the reticuloendothelial cell-forms of the splenic puncture into the following groups: (1) serosa cells, (2) pulp cells, (3) small lymphoid reticulum cells, (4) macrophages, (5) tissue mast cells, (6) plasmacellular reticulum cells.

Splenograms of normal spleens and of the various splenomegalies are presented. It is pointed out, for example, that during remission in hemolytic anemia where the only remaining symptom is splenomegaly, a splenic puncture showing many hemosiderin macrophages and the presence of individual erythroblasts without inflammatory changes, leads to the diagnosis of latent hemolytic anemia.

The text is amply illustrated with 119 figures, fifteen of which are in color.

Newark, N. J.

SAMUEL A. GOLDBERG

Diseases Transmitted from Animals to Man. Ed. 3. By THOMAS G. HULL, Ph.D., Director, The Scientific Exhibit, American Medical Association, Chicago. 571 pp., 75 illus., 67 tables. \$10.50. Springfield, Illinois: Charles C Thomas, 1947.

The medical man is greatly indebted to Dr. Hull for this up-to-date information in a field so important to the physician and the veterinarian. The author was assisted in this work by a group of fourteen contributors, who are specialists in their field. Six of them are physicians, six are veterinarians, one has both degrees and one is a doctor of philosophy. Each disease is presented with a brief historical background and with enough bacteriology, pathology and clinical data to round out the information for the average reader. The main emphasis is on epidemiology and the means by which the disease can be prevented. Naturally anyone desiring complete data on any specific disease would want to consult other works. New chapters have been added, since the last edition, on rickettsial and virus diseases. This is specific evidence of the fact that this book is up-to-date since much of this information became available only during the past war. This book should be a very valuable and useful addition to the library of every physician, veterinarian, public health worker and scientist.

East Lansing, Michigan

C. S. BRYAN

Cancer: Diagnosis, Treatment, and Prognosis. By LAUREN V. ACKERMAN, M.D., Pathologist to the Ellis Fischel State Cancer Hospital, Assistant Professor of Pathology, Washington University School of Medicine, St. Louis; and JUAN A. DEL REGATO, M.D., Radiotherapist to the Ellis Fischel State Cancer Hospital, Formerly Assistant to the Radium Institute of the University of Paris. 1115 pp., 749 illus., 42 in color. \$20.00. St. Louis: The C. V. Mosby Company, 1947.

It has become apparent during the last two or three decades, that, barring new fundamental discoveries, improvement in the treatment of cancer can be achieved mainly by the cooperative effort of teams composed of clinicians, surgeons, radiologists and pathologists. This conception has found its expression in the development of tumor clinics, mainly through the sponsorship of the American College of Surgeons. In the course of time a new specialty has begun to evolve, the cancer specialist. At first glance, this specialty seems to be inconsistent with the underlying view of tumor clinics, that is, that treatment of cancer is not one man's job. This contradiction is more apparent than real. In reality the need for teamwork remains; the new specialist is something of a general practitioner of cancer, who remains in need of special skills possessed by surgeons, radiologists and pathologists. This book is designed to serve primarily the needs of the cancer specialist, but every physician, be he general practitioner or specialist in any field, will find here a summary of up-to-date information on cancer.

The first part, comprising five chapters, deals with general aspects of cancer research (by Dr. Shimkin of the National Cancer Institute), of pathology, surgery, and radiotherapy. The fifteen chapters of the second part take up cancer of skin, respiratory and upper digestive tract, thyroid, mediastinum, digestive tract, genito-urinary tract, male genital organs, suprarenal glands, female genital organs, mammary gland, bone, soft tissues and eye, Hodgkin's disease, and leukemia. Tumors of the brain have not been included. The topics of the second part cover the following phases of every subject: anatomy (with special emphasis on lymphatics), incidence and etiology, pathology, clinical evolution, diagnosis, treatment, prognosis, and selected references.

Space allotment is quite uneven, for instance, 282 pages have been given to cancer of the respiratory system and upper digestive tract, and only 48 to cancer of the breast, and 42 to cancer of the bone. The disparity is explained in the preface "by the desirability of information in certain rare subjects and by the necessity of greater knowledge on some aspects of the more curable forms of cancer".

The presentation is excellent. Fine editing is evident on every page. The illustrations are instructive and well integrated with the text. The correlation of photographs of roentgenograms alongside of photographs of gross pathologic findings is carried out throughout the book to great advantage. The same holds for the many well executed diagrammatic drawings.

The pathologist will find in this volume good treatment of gross pathologic findings, in addition to much other invaluable information. He can ill afford to miss it.

Chicago

I. DAVIDSOHN

Anatomia Patologica de las Inflammaciones Especificas. By RAMIRO PICO DUNI, Docente libre de la catedra de anatomia y fisiologica patologicas de la Facultad de Ciencias Medicas de Buenos Aires, jefe del laboratorio de anatomia patologica del Hospital Argerich. 189 pp., 62 figs., 6 tables. Buenos Aires: Lopez and Etchegoyen, S.R.L., 1946.

This is one of a collection of monographs which honors the name of the late Pio Del Rio Hortega. The monograph covers syphilis, leprosy, actinomycosis and tuberculosis in the order named. In very readable form, especially for students, the author has marshaled the authoritative information on these four specific infectious diseases. He has limited himself to the etiology and underlying biologic and anatomic pathology omitting diagnostic procedures and therapy. His treatment of these has been didactic and for clarity and brevity he has minimized the presentation of conflicting opinion. There is little that is actually new.

In the section on leprosy, he has analyzed the various classifications of the clinical disease and has oriented them with the underlying histopathologic and immunologic findings which form the basis of the "South American Classification". This classification, like most others, recognizes three groups: (1) lepromatous; (2) tuberculoid; and (3) noncharacteristic, and each of these has three subgroups, *viz.*, cutaneous, nervous and cutaneonervous.

In the chapters on tuberculosis, the author, in similar manner, discusses the relation of immunity and allergy to the anatomic manifestations of the disease.

The monograph is printed on a good grade of paper and most of the photomicrographs are both well chosen and well reproduced. There is a comprehensive index but there is no bibliography.

Cincinnati

WILLIAM MCKEE GERMAIN

The Murine Type of Tubercle Bacillus (The Vole Acid-Fast Bacillus). Medical Research Council Special Report Series No. 259. By A. Q. WELLS, with Notes on the Morphology of Infection by the Vole Acid-Fast Bacillus by A. H. T. ROBB-SMITH. 48 pp., 13 plates, 10 figs., 16 tables. 2 shillings. London: His Majesty's Stationery Office, 1946.

Tuberculosis in the wild vole, *Microtus agrestis*, was reported in 1937. The disease has been identified in more than 900 small mammals of four different species. Distribution of lesions, route of infection, duration of disease, bacterial characteristics, tuberculin production, antigenic structure, pathogenicity and immunization experiments with the vole bacillus are described in detail. The vole bacillus produces a resistance to subsequent infection with virulent tubercle bacilli in animals. The resistance in guinea pigs is not immunity, according to Wells, but the time interval between onset of infection and death is increased considerably. The degree of resistance in cattle has not yet been determined. To plan a controlled experiment in tuberculosis vaccination in man is so difficult as to be virtually impossible. The relative merits of BCG and the vole bacillus as antigen are discussed. It appears that the latter is incapable of producing other than localized disease; that the safety of BCG appears to be beyond dispute; that the local reaction from the vole bacillus in man is dependent on the depth of injection (a multiple puncture apparatus is described) that the reaction resembles the local reaction to BCG and in no case has the reaction been severe; that the vole bacillus produces tuberculin sensitivity on injection; that the degree of induced resistance appears to be greater in guinea pigs for the vole bacillus than for the BCG in the early stages of tuberculosis and about equal in the later stages; that in cattle the vole bacillus may confer more resistance than BCG; and that no evidence is available in man. The vole bacillus has not been made avirulent *in vitro* but possesses naturally a suitable virulence for vaccination, and virulence for voles can be maintained indefinitely by passage. Robb-Smith contributes a morphogenetic analysis of the tissue changes induced by mycobacteria in the vole.

For those interested in vaccination with the vole bacillus and an authoritative study of this new member of the *Mycobacterium* group (the first described for nearly forty years), this contribution will be of special benefit.

Denver

H. J. CORPER

Standard Methods of the Division of Laboratories and Research of the New York State Department of Health. Ed. 3. By AUGUSTUS B. WADSWORTH, M.D. 990 pp., 105 figs., 77 tables. \$10.00. Baltimore: The Williams and Wilkins Company, 1947.

Changes have been made in nearly all sections in the third edition of this book. One of the more important revisions has been made in the routine methods for the serodiagnosis of syphilis. Here, detailed procedures are given for the preparation and standardization of the new cardiolipin-lecithin-cholesterol antigen. A chapter on the estimation of potency by biologic assay is added.

The text is divided into 75 chapters and an appendix. The first twelve chapters are devoted to general laboratory procedures including general bacteriologic technic, preparation and use of stains and reagents, care and breeding of laboratory animals, maintenance of type culture collection, determination of hydrogen-ion concentration, methods of quantitative chemical analysis and precision tests, methods for volumetric calibration and microvolumetric measurement, a discussion of spectroscopic methods and an outline for biologic assay. Chapters 13 through 16 are devoted to the preparation of glassware and bacteriologic mediums. Chapters 17 through 38 take up the methods used in the diagnostic laboratories of the New York State Department of Health. Their bacteriologic, serologic, parasitic, chemical, hematologic and histologic procedures are described. A short chapter is included

on examination for incitants of food poisoning. Chapter 30 gives a detailed account of serologic tests for evidence of syphilis, tuberculosis and gonococcus infections. From the standpoint of the public health laboratory, chapters 39 through 42 give helpful outlines for the examination of water and sewage, milk and cream and of samples concerned with restaurant sanitation. The final chapters are devoted largely to the methods used in the antitoxin, serum and vaccine laboratories.

Provisions of the New York State laws and sanitary code relating to approved laboratories, and provisions of the public health law and sanitary code relating to pathogenic microorganisms and viruses are listed in the appendix. Outfits, together with blank forms and directions, are described for the submission of specimens. Postal laws and regulations relating to shipment of various specimens are also given. The index is complete.

The paper and print are of good quality, the headings and subheadings are helpful, while the direct style of the text makes it easily understood and readable. As a reference book for the large hospital laboratory and public health laboratory, this book is ideal and can be recommended without reservation.

Forensic Medicine. By KEITH SIMPSON, M.D., Lecturer in Forensic Medicine to Guy's Hospital, London, Examiner in Forensic Medicine, University of London. 334 pp., 114 figs. \$4.50. Baltimore: The Williams & Wilkins Company, 1947.

This concise textbook "designed to provide a brief and essentially practical guide, from an English school, to current teaching in forensic medicine", has been well done. It is attractive, adequately illustrated and well written. At appropriate places in the text, there are useful tables, technics and tests which are outlined briefly and clearly. A number of illustrative cases from the author's own experience, usually set down in a few sentences, serves to increase the interest of the reader. A section, consisting of 86 pages, deals rather fully with all types of poisons. It includes discussions of sources of poisons, signs and symptoms, treatment, fatal doses and autopsy appearances.

Like so many of the English writers of medical books, the author has a delightful style which lures the reader on. At the same time, the material is presented in a logical, lucid and factual manner. This is one of the better books on the subject of medicolegal pathology and is highly recommended.

The Pharmacopoeia of the United States (The United States Pharmacopoeia), Thirteenth Revision, U.S.P. XIII. Prepared by the Committee of Revision and published by the Board of Trustees by authority of the U. S. Pharmacopoeial Convention meeting at Washington, D. C., May 14 and 15, 1940. 957 pp., 12 figs., 23 tables. \$8.00. Easton, Pennsylvania: Mack Printing Company, 1947.

The first portion of this revision, occupying 107 pages, includes a listing of the names of all persons and organizations officially concerned with the revision and the history of the United States Pharmacopoeia. It also includes articles added to the U.S.P. XIII and articles official in the U.S.P. XII, but not admitted to the U.S.P. XIII. The main portion of the Pharmacopoeia contains general notices applying to the Standards of the U.S.P., and some 600 pages devoted to monographs on drugs, chemicals and preparations. An important innovation is the listing of each drug under its official English title. Below the title follow the Latin name, abbreviation, popular name, chemical and structural formulas, molecular weight, descriptions, solubilities, identification, content of ash, foreign organic material and moisture, and, where indicated, such information as optical rotation, insoluble residue, loss on drying, residue on ignition, melting range, heavy metals, water absorption, clarity of dilution, specific gravity, assay, directions on packaging and storage, other cautions to be observed and average dose by various methods of administration.

A very valuable section, comprising 114 pages, then follows on general tests, processes and apparatus dealing with such subjects as alcohol determination, anti-anemia preparations, arsenic tests, ascorbic acid assay, bacteriologic examination of gelatin, carbon dioxide absorbency of soda lime, chromic acid cleansing mixture, clarity of parenteral solutions,

suggested types of glass containers for preparations for injection, standards for light transmission of containers, diameters of sutures, dithizone test for lead, emulsions, extracts, fats and fatty oils, fiber length of cotton, fineness of powder, hardness of soda lime, heavy metals test, general identification tests, official medicine dropper, melting range or temperature, nicotinic acid or nicotinamide assay, Kjeldahl method and semimicro-Kjeldahl method, optical rotation, pyrogen test, refractive indexes of some U.S.P. crystalline substances, riboflavin assay, sterility tests for liquids and solids, sterilization processes, tensile strength determination, thiamine assay, turbidimetric tests, methods for sampling and analysis of vegetable and animal drugs, Vitamin A and Vitamin D assays.

Another section of 57 pages deals with reagents, test solutions, colorimetric solutions, indicators, volumetric apparatus and solutions, hydrogen ions and pH. The final section, comprising 49 pages of tables, includes atomic and molecular weights, thermometric equivalents, calibration of glass measuring apparatus, weight and volume relations, equivalents of weights and measures, equivalents of linear measures and conversion tables.

The Pharmacopoeia represents a vast store of up-to-date authentic chemical data. If medical workers acquired an appreciation of the extensive amount of useful information contained in the Pharmacopoeia, many of them would consult it more frequently.

NEWS AND NOTICES

CONFERENCE ON NOMENCLATURE OF THE Rh FACTORS

The Surgeon General of the United States Public Health Service has appointed an advisory board, whose function will be the designation of proper names for the several anti-Rh blood typing serums licensed under the Biologics Law. The goal is to have the board recommend a system of nomenclature which will be adequate to cover this phase of human genetics, and of equal importance, one which can be readily adapted to clinical use, medical teaching and laboratory diagnosis. The members of the board are: Dr. William B. Castle, Jr., Professor of Medicine, Harvard Medical School; Dr. Maxwell M. Wintrobe, Professor of Medicine, University of Utah; and Dr. Laurence H. Snyder, Professor of Medical Genetics, University of Oklahoma.

A conference on this subject was held on October 20 and 21, 1947, at the Hotel Statler, Washington, D. C. The following speakers were listed on the program: Dr. M. V. Veldee, Dr. Laurence H. Snyder, Dr. Alexander S. Wiener, Dr. Philip Levine, Dr. H. H. Strandskov, Dr. Joseph M. Hill, Dr. Louis K. Diamond, Dr. Elmer L. DeGowin, Dr. Warren E. Wheeler and Dr. Ernest Witebsky.

INTERNATIONAL CONFERENCE OF CLINICAL BIOLOGY

The First International Conference of Clinical Biology is scheduled to be held November 20, 21 and 22, in Paris, under the auspices of the Société Française de Biologie Clinique. The morning sessions will be devoted to observing demonstrations at various hospitals throughout Paris, while the afternoon sessions will be held at the Hôpital des Enfants Malades. Dr. L. Durupt is general secretary of the Society.

PENNSYLVANIA ASSOCIATION OF CLINICAL PATHOLOGISTS

A meeting of the Pennsylvania Association of Clinical Pathologists, was held at the Hotel Roosevelt, Pittsburgh, September 12 to 14, inclusive. A scientific session was held September 13, at 2:00 P.M., and the following papers were presented: Thromboembolic Diseases—Experimental and Clinical Aspects, Dr. K. Y. Yardumian, Dr. Richard E. Rosenfield and Dr. Harold S. Tuft; Studies of the Reticulum in Carcinoma of the Skin, Dr. Paul Gross; Important Considerations in Graduate Laboratory Training, Dr. Samuel R. Haythorn; and Observations on Tissue in Patients Treated with Nitrogen Mustard, Dr. G. H. Fetterman and Dr. G. J. Kastlin. The officers elected for the coming year are: Dr. Frederick O. Zillesen, Easton, president; Dr. Theodore R. Hembold, Pittsburgh, vice president; and Dr. Henry F. Hunt, Danville, secretary-treasurer.

MINNESOTA SOCIETY OF CLINICAL PATHOLOGISTS

The annual meeting of the Minnesota Society of Clinical Pathologists was held in Duluth on July 1, 1947, during the annual session of the Minnesota State Medical Association. Nineteen members and several guests were in attendance.

The following scientific program was presented: Bone Marrow Studies, Dr. Gertrude Lorna Pease, Rochester; Parathyroid Adenoma, Dr. Harold Joffe, Duluth; Some Problems in Diagnosis of Coccidioidomycosis, Dr. Lyle Weed, Rochester; The Use of Xanthidrol to Demonstrate Uremia in Tissue Sections, Dr. W. V. Knoll, Duluth; Carcinoma (Cylindroma Type) of Bartholin's Gland of 23 Years' Duration, Dr. George Sayre, Rochester; Tumors of the Parotid and Submaxillary Region, Dr. T. E. Ludden and Dr. Edith Parkhill, Rochester.

This program was followed by The A. H. Sanford Lecture, which was delivered by Dr. E. T. Bell, Minneapolis, on Pathology of Diabetes, at the Duluth Armory, before the general session of the Minnesota State Medical Association. The A. H. Sanford Lectureship, in honor of Dr. A. H. Sanford, Rochester, was established by the Society and dedicated to the Minnesota State Medical Association.

In the evening, an informal dinner party was tendered by Dr. and Mrs. A. H. Wells at their home, and a delightful social hour was enjoyed.

The following officers and honorary members were elected: President, Dr. A. H. Baggenstoss, Rochester; Vice President, Dr. Robert Hebble, Minneapolis; Secretary-Treasurer, Dr. Kano Ikeda, Saint Paul; Councilors, Dr. A. H. Wells, Duluth; Dr. Nathaniel Lufkin, Minneapolis; and Dr. G. G. Stilwell, Rochester; Honorary Members, Dr. E. T. Bell, University of Minnesota; Dr. B. J. Clawson, University of Minnesota; Dr. A. H. Sanford, Rochester; and Dr. William C. MacCarty, Rochester.

The following resolution was framed and presented to the Council of the Minnesota State Medical Association:

1. WHEREAS, It has come to the attention of the Minnesota Society of Clinical Pathologists, that the House of Delegates of the Minnesota State Medical Society has passed a resolution requesting the State Board of Health to perform tests for the Rh factor; and
2. WHEREAS, It is felt that the practicing clinical pathologists in the State of Minnesota already have the experience, knowledge and facilities available for the adequate performance of these tests; and
3. WHEREAS, They are perfectly willing to perform such tests free of charge for indigent patients; and
4. WHEREAS, This sets a precedent for the performance of laboratory work by the State Board of Health outside of the field of contagious disease; and
5. WHEREAS, It is felt that income from such work would act as a partial inducement for pathologists to establish practice in communities where their services are not at present available; and
6. WHEREAS, The value of the tests concerning Rh factor rests in their proper interpretation by the clinical pathologist as a consulting physician; therefore,

BE IT RESOLVED, That we, the Minnesota Society of Clinical Pathologists can not express approval of the resolution passed by the House of Delegates. We respectfully solicit the interest of the Council in our viewpoints, should their future activities have any bearing on the action of the Board of Health in this important matter.

UNIVERSITY OF NEBRASKA COLLEGE OF MEDICINE

The following changes have been made in the faculty of the College of Medicine, University of Nebraska: Dr. Harold E. Eggers has retired as Chairman of the Department of Pathology and Bacteriology; Dr. J. P. Tollman has been appointed as Professor of Clinical Pathology and Chairman of the Department of Clinical Pathology and Bacteriology; Dr. John R. Schenken has been appointed Professor of Pathology and Acting Chairman of the Department of Gross and Microscopic Pathology; Dr. Pliney Allen has been appointed Assistant Professor of Pathology and Dr. Robert M. Allen has been appointed Assistant Professor of Bacteriology.

OKLAHOMA ASSOCIATION OF PATHOLOGISTS

The Oklahoma Association of Pathologists has announced the election of the following officers: Dr. Béla Halpert, Oklahoma City, president; Dr. Emil Palik, Tulsa, vice president; Dr. J. N. Owens, Jr., Shawnee, Secretary-Treasurer; and Dr. W. F. Keller, Member of Council.

DEPARTMENT OF SELECTED ABSTRACTS

Any reader of the Journal who wishes to serve as a contributor to the Department of Selected Abstracts may communicate with the editor, listing the journals which are available to him and stating his special field of interest in clinical pathology or pathologic anatomy. There is no monetary compensation, but the name of the abstracter is published.

PERSONAL

Mr. Edward B. Ellis, formerly of the New York Memorial Hospital, is now connected with the Technicon Company, 215 East 149th Street, New York City. Mr. Ellis was associated with the late Dr. James Ewing of Memorial Hospital for over forty years.

TECHNICAL SECTION

FROZEN THROMBOPLASTIN EXTRACTS IN PROTHROMBIN DETERMINATIONS*

JOSIAH FULLER, M.D.

From the Laboratories, Veterans Hospital, White River Junction, Vermont, and Mary Hitchcock Memorial Hospital, Hanover, New Hampshire, and Dartmouth Medical School

Dicumarol may be used safely and successfully when the dosage is guided by reliable prothrombin determinations, as has been emphasized by Barker and his colleagues^{3, 4, 5} and others.^{6, 15, 22} In 1938, Quick¹⁶ described a prothrombin dilution curve and recently, Hurn *et al.*^{8, 9} and Aggler *et al.*² presented a statistical evaluation of the procedure.

For practical clinical use, a fairly large supply of thromboplastin of constant potency is necessary to obviate the necessity of making new prothrombin dilution curves frequently, as the construction of these curves requires that prothrombin times be done on serial dilutions of at least five plasmas, as shown by Aggler and his colleagues.²

I agree with Aggler¹ who found commercially available thromboplastin unsuitable. For this reason I endeavored to produce my own thromboplastin.

The method most often recommended^{2, 9, 11, 14, 17} of storing the dried powdered brain thromboplastin under a vacuum usually below 20 C. calls for the weighing out of small quantities of the powder for each test. This is done immediately after preparation with aliquots stored in evacuated ampules, or it is done before each test. I have encountered difficulties in getting a powder sufficiently homogeneous so that each aliquot is of the same potency. The construction of evacuated ampules is a technic that is not easily adaptable to the usual clinical laboratory. It is also necessary for the technician to extract the powder with saline each time, and I have found that strict attention must be paid to this process or appreciable differences in the potencies of the extracts will result. Certainly a more simplified and, perhaps a more reliable technic, is desirable for a test such as this which is used so frequently and is so important in Dicumarol therapy.

Storing the thromboplastin as the saline extract would obviate some of the objections, but the instability of saline extracts is well known. Kazal *et al.*¹² were able to prolong the activity by adding antioxidants and antiseptics. Preservation by the lyophil technic of Folsdorf and Mudd,⁷ as described by Souter and Kark,¹³ and by Souter, Kark and Taylor,²⁰ and Kazal and Arnow¹¹ would seem to be the ideal method. A lyophil apparatus, however, is not standard equipment in most

* Received for publication, July 12, 1947.

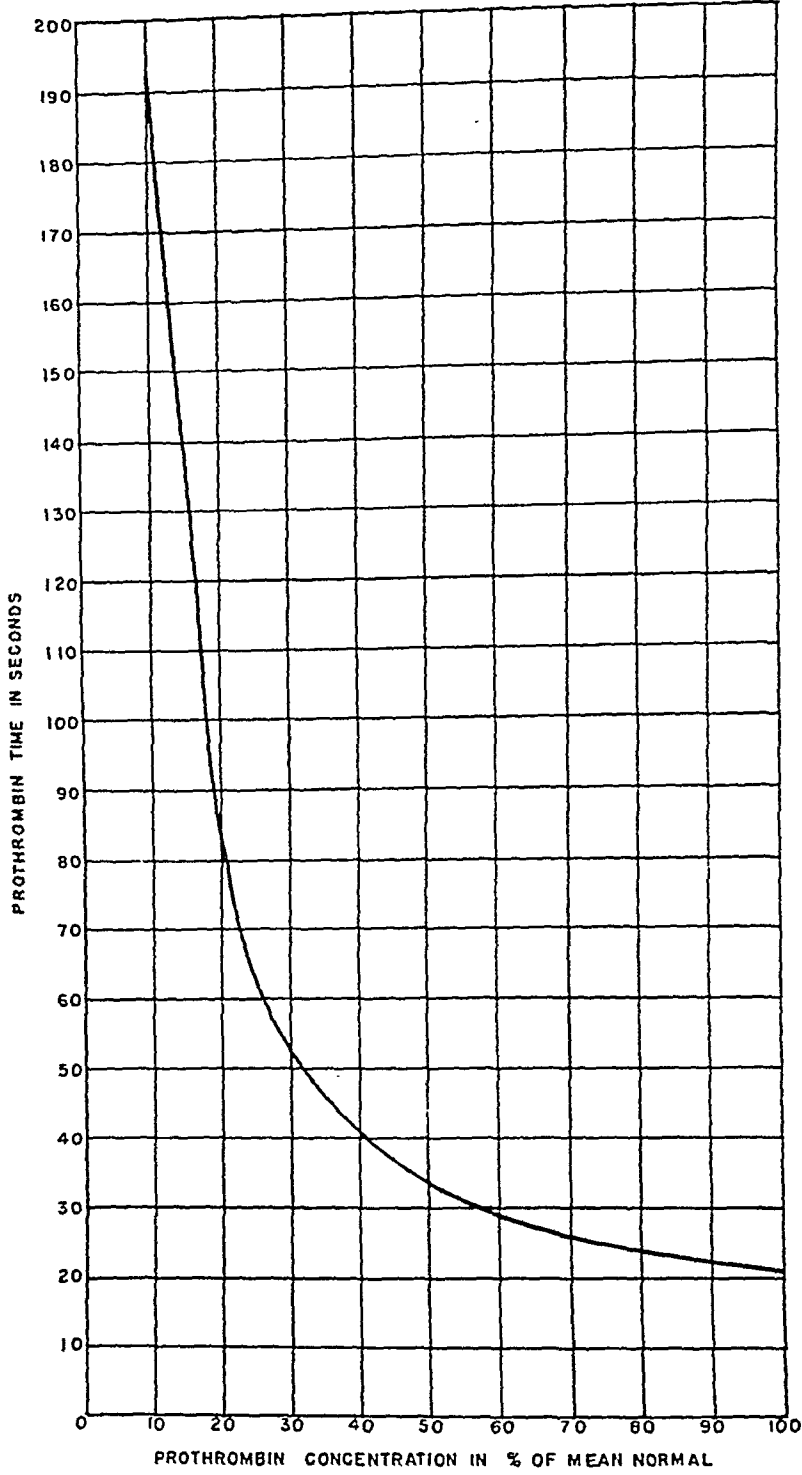


FIG. 1. Prothrombin dilution curve for a frozen thrombo-
plastin extract.

hospitals, although the lyophil technic may prove to be the most satisfactory way of producing thromboplastin industrially.

Preserving saline extract by freezing was described in 1940 by Irving,¹⁰ who thawed and refroze a saline extract periodically for nine months without any appreciable change in the potency as measured on undiluted normal control plasmas. Since his is the only report I have found on this simple but useful technic, my experiences may be worth recording.

METHOD

Thromboplastin was prepared by a method derived from the Quick¹⁶ acetone extraction technic, as modified by Aggler *et al.*² for use on human brain, and the technic for horse brain of Kazal *et al.*¹⁴ Human cerebral hemispheres were stripped of pia and blood vessels. Small portions were macerated under acetone in a mortar with a pestle, using as many changes of acetone as necessary to obtain a fine, white, granular powder, which was then dried on a suction filter. Only small portions of the hemispheres were dehydrated at a time so that the dehydration process could be completed in from fifteen to twenty minutes. It was necessary to use about five times the volume of acetone as there was brain tissue and to change the acetone three or four times.

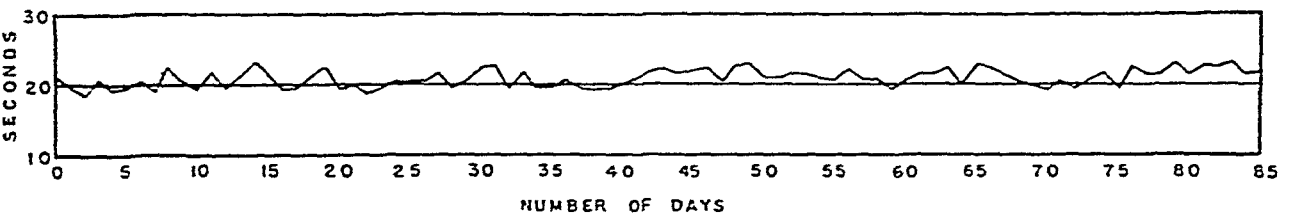


FIG. 2. Prothrombin time of controls in the daily use of a frozen thromboplastin extract.

The powder was added to 0.85 per cent saline solution making a 6 per cent suspension. This was extracted at a temperature of 55 C. in a water bath for fifteen minutes with constant stirring. The suspension was then centrifuged at 1000 R.P.M. for two or three minutes to bring down the large particles. The supernatant was placed in test tubes in 2.5 ml. amounts and tightly stoppered. The lot was "quick-frozen" in a freezing unit and then stored in the frozen state. Before use, this frozen extract was thawed at 37.5 C. for thirty minutes. The tube was agitated momentarily every five minutes, while thawing.

This whole procedure, except the freezing, can be accomplished with no special apparatus other than that found in any good clinical laboratory. Freezing units are quite generally available either in standard refrigerators or regular freezers.

The thromboplastin was standardized by doing prothrombin time determinations (Quick method¹⁵) on serial saline dilutions of plasma of five known normal persons using five of the above tubes as recommended by Aggler *et al.*² This produced the prothrombin dilution curve shown in Figure 1.

This thromboplastin was then used in the performance of routine prothrombin determinations in our laboratory. They were reported in percentage of mean normal as well as time in seconds compared with the control. Figure 2 represents the prothrombin times of the controls used each day. These controls were taken

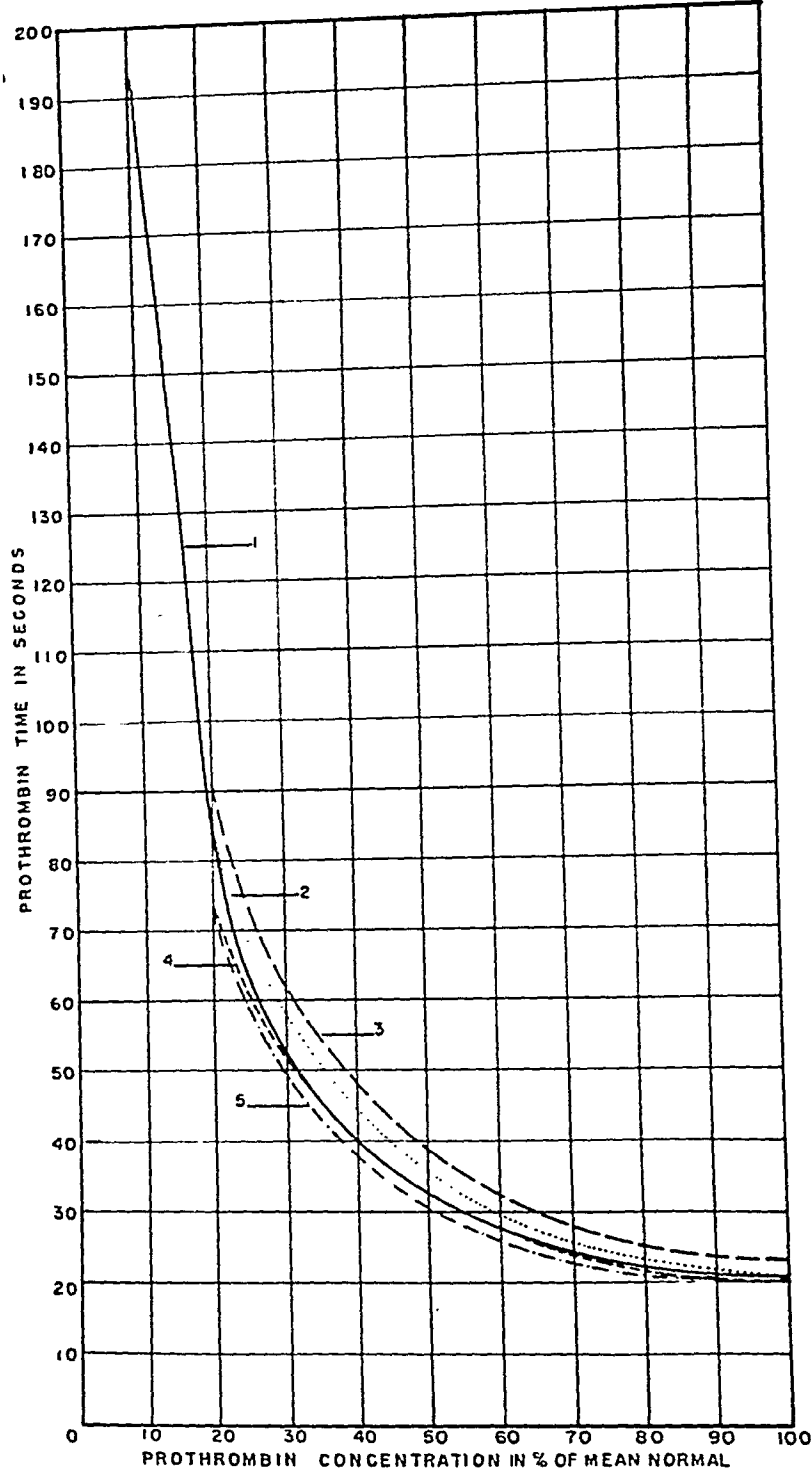


Fig. 3. Comparison of periodic modified prothrombin dilution curves with the original. (1) Original; (2) 5 days; (3) 23 days; (4) 37 days; (5) 90 days.

at random from any available known normal person. They were used as a check on the test and as a daily check on the stability of our extract. It will be seen

that during the eighty-five day period there was no definite trend indicating a change in potency.

Hurn *et al.*⁹ and Aggler,¹ however, have shown that thromboplastins giving similar prothrombin times on undiluted plasma may vary widely on diluted plasma thereby giving widely divergent results on prothrombinopenic individuals. It was conceivable that a qualitative change, not brought out by the daily controls, could have taken place in the thromboplastin. In order to determine this, I constructed, at intervals during the period of use of this thromboplastin, modified prothrombin dilution curves on one individual using 100 per cent, 30 per cent, and 20 per cent concentrations of plasma. From Figure 3 it will be seen that during the ninety days of observation there was no trend indicating a loss of potency. My statistical data have been critically examined, and the slight differences noted on the chart were found to be well within the limit of error for the determinations as calculated from the data used to construct the original curve.

Recently one lot of frozen thromboplastin suddenly, after one hundred and twelve days, exhibited a slight loss in potency. Previously it had demonstrated stability comparable to that reported here and the reason for the sudden change is not at present apparent.

CONCLUSION

Frozen saline thromboplastin extracts are a convenient source of thromboplastin for the Quick method of prothrombin determinations, and they have been shown to retain their potency for at least three months.

Acknowledgments. I am indebted to Alan Mather, Ph.D., Assistant Professor of Biochemistry, Dartmouth Medical School, for first suggesting to me the freezing of thromboplastin as a method of preservation, and to James F. Crow, Ph.D., Assistant Professor of Preventive Medicine and Parasitology, Dartmouth Medical School, for a statistical evaluation of the data.

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A RAPID METHOD FOR THE EVALUATION OF MICROBIOLOGIC TESTS*

WALTER KOCH, M.D.

From the Department of Hygiene, Hebrew University, Jerusalem, Palestine

Since the introduction by Snell and Strong³ of a microbiologic test for the assay of riboflavin, the same principle has been adopted for the estimation of other vitamins and amino acids.^{2, 4} A standard curve is prepared by plotting known amounts of the vitamin added to the broth against turbidity caused by bacterial growth. Unknown quantities are determined by interpolation. Generally, the standard curve is derived from the mean of several experiments. Its usefulness, however, is limited by day-to-day variations in the growth response of bacteria. Such variations may be due to slight changes in the composition of the medium, size and age of inoculum, and time and temperature of incubation. It is, therefore, widely accepted that a series of daily standards must be run to make the necessary correction. Since this is time-consuming, a graphic method is described which takes daily variations into account and requires that only two suitable dilutions of the standard be run daily. The necessary daily correction is determined within a few seconds. The method has been tested in the assay of riboflavin and nicotinic acid and should be useful in other similar tests.

METHOD

A nomogram is constructed and its centering point changed according to the daily growth response.

Table 1 shows the amounts of nicotinic acid in micrograms contained in 10 ml. of medium and the mean values of the corresponding colorimetric readings.¹

The procedure is then as follows. Two parallel perpendicular lines are drawn, about four and one-half inches apart (Fig. 1). A point, C, is chosen (Fig. 1) equidistant from both lines. The amounts of nicotinic acid listed in Table 1 are plotted on a logarithmic scale on the right hand line. As shown in Table 1, 0.005 microgram of nicotinic acid gives a mean reading of 83. Therefore, a line (broken line, Fig. 1) is drawn through point 0.005 and point C; its point of intersection with the second parallel line is designated 83. Similarly, a line drawn from point 0.01 through point C will locate the point on the left line which corresponds to a mean reading of 79; the points on the left line will be located for the other readings in the same way. The line between 79 and 83 is divided into four equidistant spaces, that between 72 and 79 into seven equidistant spaces, etc. (Fig. 2). For greater convenience, the logarithmic scale is also subdivided and extended for readings obtained with amounts of nicotinic acid above 0.10 microgram. The nomogram is now covered with a piece of transparent paper or washed x-ray film. On a similar strip of transparent paper, the logarithms of 1, 2, 3, 5, 6 and 10 are marked off (Fig. 3), since 0.1, 0.2, 0.3, 0.5, 0.6 and 1.0 ml. of a dilution of the unknown have been added to the broth in this case.

* Received for publication, May 22, 1947.

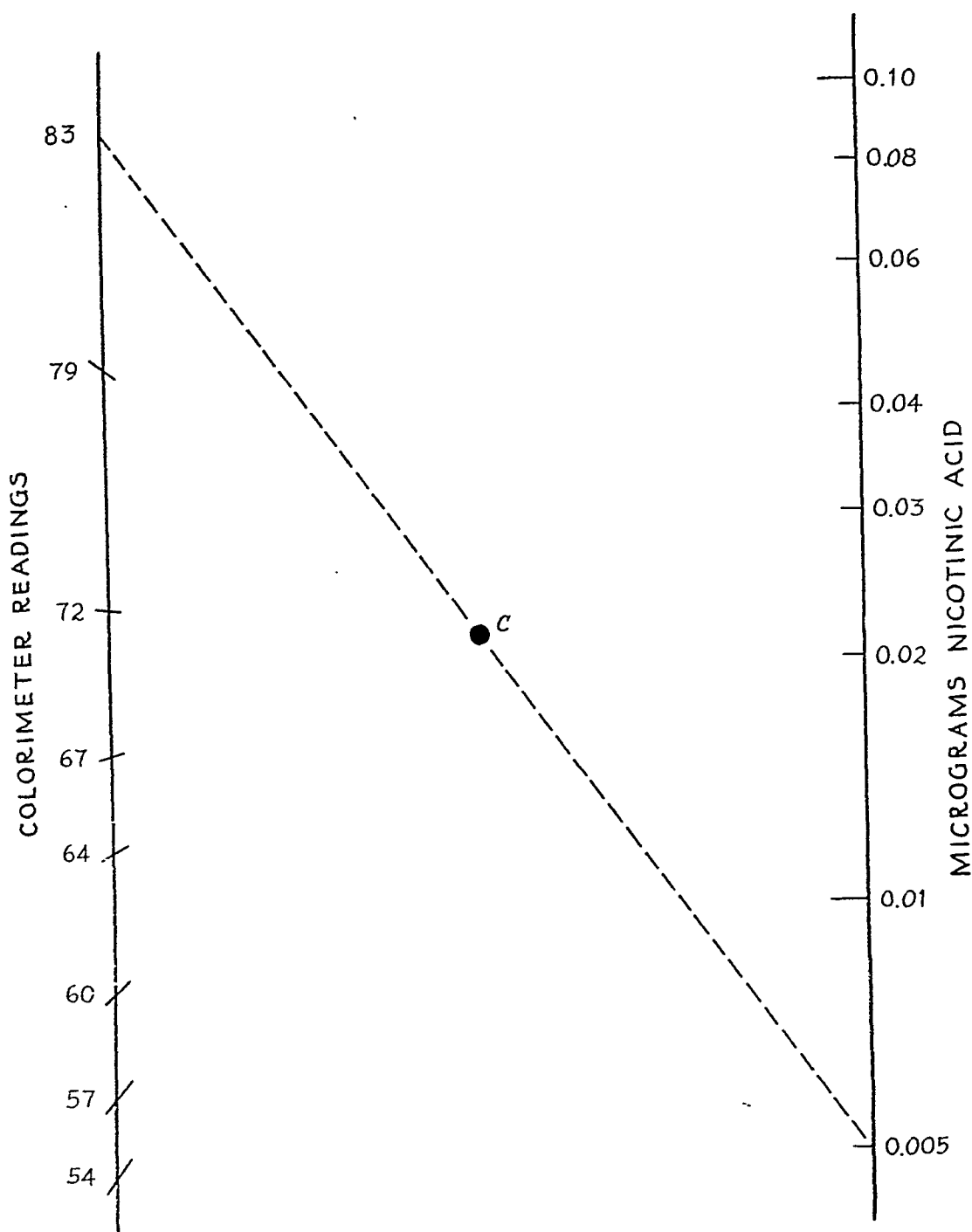


FIG. 1. Plotting of mean growth responses against colorimeter scale by drawing lines through centering point C (see Table 1).

The nomogram developed above indicates the mean growth response, but does not, however, indicate the daily growth. Table 2 and Figure 4 (hollow circles)

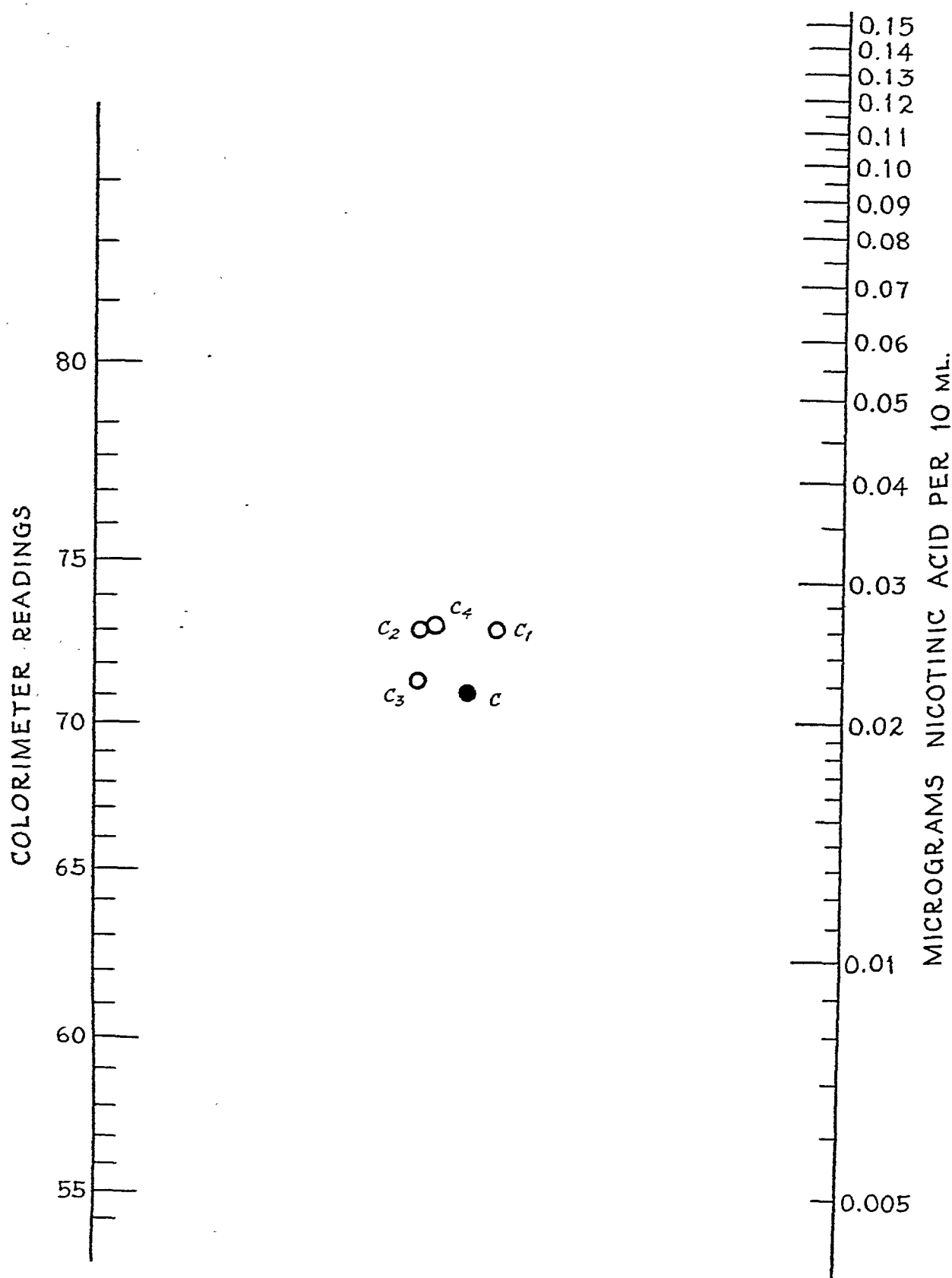


FIG. 2. Centering point C (full dot) is the same as in Figure 1 and represents the mean growth response. C_1 , C_2 , C_3 and C_4 show growth responses on four different days. C_1 represents the point of intersection of a line joining points 0.02 and 76 and a line joining points 0.06 and 62 (see Table 2). C_2 represents the point of intersection of a line joining points 0.02 and 75 and points 0.06 and 65.5 (see Table 3). C_3 represents the point of intersection of a line joining points 0.02 and 72.25 and points 0.06 and 62.75 (see Table 3). C_4 represents the point of intersection of a line joining points 0.02 and 75.25 and points 0.06 and 65 (see Table 3).

show how much the daily growth response of the standard sets differs from this mean. By comparison with Table 1, it can be seen that growth was rather poor on the day shown in Table 2. The readings of any day under consideration can, however, be made to fit the nomogram by changing the centering point, C, in the nomogram. The positions of new centering points, C₁, C₂, C₃ and C₄, valid for each of four different days, are determined from the response as shown by standards of 0.02 and 0.06 microgram nicotinic acid. These two amounts have been

TABLE 1
RESPONSE OF PROTEUS HX19 TO NICOTINIC ACID

MICROGRAMS NICOTINIC ACID IN 10 ML. OF MEDIUM	MEAN COLORIMETRIC READINGS
0.005	83
0.01	79
0.02	72
0.03	67
0.04	64
0.06	60
0.08	57
0.10	54

TABLE 2
AN ILLUSTRATION OF THE DECREASED ERROR BASED ON THE CENTERING POINT OF THE NOMOGRAM DETERMINED BY THE DAILY GROWTH RESPONSE

MICROGRAMS NICOTINIC ACID PER 10 ML.	COLORIMETRIC READINGS	PERCENTAGE ERROR WHEN USING:	
		Mean Center C	Daily Center C ₁
0.01	82	40.0	0.
0.02*	76	33.0	0.
0.03	72	33.3	6.7
0.04	69	36.3	12.5
0.06*	62	20.0	0.
0.08	58	9.4	7.5
0.10	55	6.4	8.0
Averages.....		25.5	4.95

* Values of the standard used to locate point C₁.

chosen as they represent the limits of the useful range. By connecting point 0.02 with point 76 of the colorimetric scale, and drawing a similar line between point 0.06 and 62, an intersection point, C₁, is found (Fig. 2). Using C₁, instead of C, the mean percentage error of the unknowns run simultaneously drops from 25.5 to 5 per cent and no calculation is required (Table 2).

PRACTICAL APPLICATION

In order to determine the appropriate centering point for each assay, two dilutions of the standard (0.02 and 0.06 microgram nicotinic acid) are run with every

batch of unknown samples. The intersection point, C_1 , is marked in ink on the x-ray film. Now the samples proper are estimated, *viz.*, urine diluted 1:20 gives a reading of 73 for 0.3 ml. and of 63 for 0.6 ml. Using C_1 , the amounts of nicotinic acid are 0.026 microgram and 0.055 microgram, respectively. Both of these points are marked in ink. In order to obtain the amount contained in 0.1 ml., the first figure has to be divided by 3, the second by 6. This can be conveniently done by means of a piece of transparent paper (Fig. 3). One-sixth of 0.055 is found to be 0.0093, while one-third of 0.026 is 0.0087. By inspection we interpolate 0.009 as the average value. This figure times 20 (the dilution) gives a result of 1.8 micrograms per ml. of urine. Since in most cases different dilutions of the same sample do not yield exactly the same results, one must try to base the answer on findings obtained within the useful range. Consequently the urine from patients treated with massive doses of nicotinic acid has to be diluted approximately 1:60 or 1:120 instead of 1:20 as stated above.

MILLILITERS OF UNKNOWN ADDED

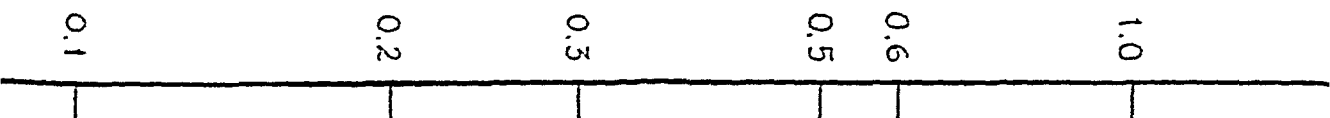


FIG. 3. This scale may be shifted along the scale marked "micrograms nicotinic acid" in Figure 2 like the slide in a slide rule bringing different amounts of the added unknown to unity.

Table 3 shows three assays of various urines. The second urine was from a treated patient and the third was rather low in nicotinic acid. As higher values were anticipated in the treated patient, most of the readings were obtained from a dilution of 1:80. The same table also shows the other extreme, urine containing little nicotinic acid. As normal values were expected the higher dilutions fell outside the range. In order to test the accuracy of the proposed method, the results derived from a complete, simultaneous set of standards is assumed to have an error of zero. These and similar assays are summed up in Fig. 4. Taking 5 per cent as the limit of possible error,* 60 per cent of all assays by this method, fall within this limit, but only 13 per cent fall within this limit when the mean standard curve is used without allowance for day-to-day variation. Ninety-two per cent of all assays by this method will have an error of less than 10 per cent, as compared with 26 per cent falling within this limit if a mean curve is used. The average percentage error of each method is 4.81 per cent and 21.0 per cent, respectively.

DISCUSSION

Certain errors are inherent in this method. In the sample to be tested, unknown substances may occur which inhibit or promote growth. Such substances change the slope of the growth response curve, as pointed out by Wood,⁵ produc-

* This is a rather stringent limit as Wood⁵ states that a difference of plus or minus 10 per cent among dilutions of the unknown "... is usually regarded as reasonable agreement", when using a simultaneous complete standard curve.

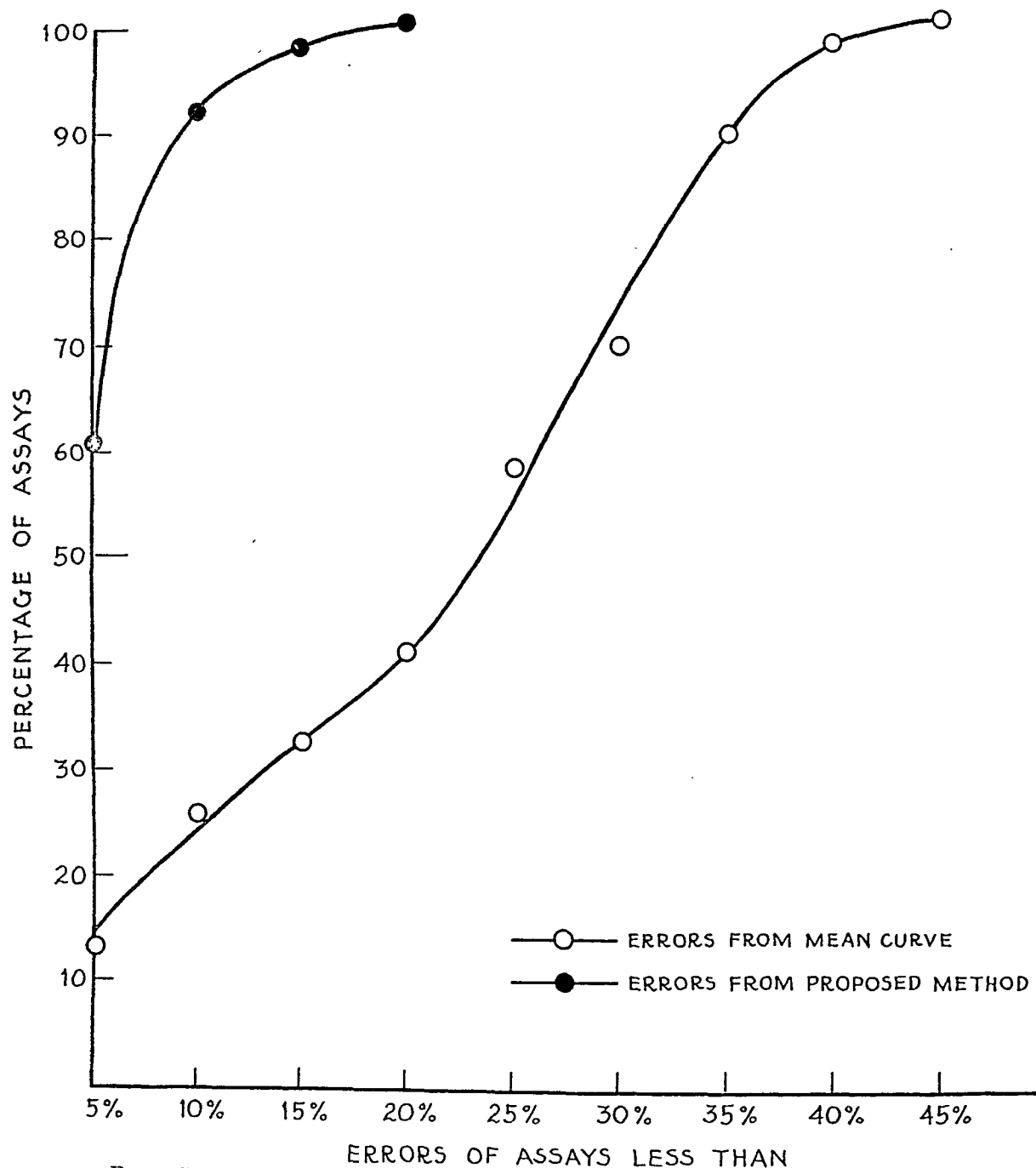


FIG. 4. Cumulative percentage frequency. Ordinate shows percentage of assays having an error smaller than that indicated in the abscissa.

ing varying results for different dilutions of the sample. The more dilute the sample, the smaller this error will be. Another difficulty is the establishment of a correct result to serve as a check for the proposed method. Several determin-

ations of each point would be necessary to determine an accurate daily standard curve, which should be a straight line. However, the three or four tests for each of the two points necessary in this method are easily performed. Since no curve has to be drawn and since the results are read from a nomogram, at least one-quarter of the total labor is saved by the method described.

SUMMARY

An adjustable nomogram for microbiologic assay of nicotinic acid and similar substances is described. Day-to-day fluctuations of growth response are compensated for without calculations. Although only two dilutions of the standard

TABLE 3

EXAMPLES OF THREE ASSAYS ON URINES. THE ESTIMATE FROM A COMPLETE, SIMULTANEOUS STANDARD CURVE IS ASSUMED TO HAVE AN ERROR OF ZERO. THE CENTERING POINTS VALID FOR EACH ASSAY, C_2 , C_3 AND C_4 , ARE OBTAINED SIMILARLY TO C_1 , AS SHOWN IN FIGURE 2

SPECIMEN OF URINE	DILUTION OF URINE	CENTER POINT FOR THIS DAY	PERCENTAGE ERROR OF:	
			Mean Standard	Proposed Method
Normal.....	1:20	C_2	27.4	0.
Treated.....	1:20 and 1:80	C_2	6.4	4.1
Graves' disease, low in nicotinic acid.....	1:20	C_4	21.0	2.5

are used in order to establish the correction, the accuracy achieved is sufficient for most purposes. Compared with a conventionally drawn semilogarithmic daily standard curve, with an error of about 20 per cent, the present method has an error of only about 5 per cent. Different dilutions are brought to unity by means of an additional scale.

Acknowledgment. The author is indebted to Dr. N. Grossowicz for putting at his disposal unpublished data of a test for nicotinic acid.

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SIMPLIFIED TECHNIC FOR CULTURE FROM BLOOD OF ENTERIC GROUPS OF BACTERIA*

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The ordinary procedure of blood examination for the typhoid-paratyphoid group of organisms consists of agglutination reactions carried out with a known serum against a bacteriologic culture preferably made from the blood clot. The advantage of the "clot culture" over the whole blood culture lies in the absence of serum which may contain the patient's specific antibodies and thus interfere with the growth of micro-organisms present.¹ In the new technic suggested here, one simple operation which is time-saving, prevents contamination and can be carried out in a small laboratory not equipped with a large centrifuge, is substituted for the centrifugation of blood and pipeting off of the serum.

TECHNIC

The blood is collected in a sterile swab tube in which the cotton swab reaches the bottom as in the ordinary tube used for diphtheria culture. The blood is allowed to clot; the clot adheres to the swab and contracts around it while the clear serum separates from it.

For bacteriologic culture, a 3 per cent glucose-bile medium is used and distributed in test tubes of approximately the same length and diameter as the swab tubes used for the collection of blood. When the blood has clotted sufficiently, the swab with its adhering clot is introduced into the culture medium tube under ordinary aseptic precautions and stoppered. The culture tube with the clot immersed in the bile-sugar solution is incubated. The serum left behind in the original swab tube is used for the Widal agglutination tests.

The blood clot dissolves in the bile medium and if glucose fermenters which acidify the medium, develop, a heavy precipitate settles, and the culture should be further examined. In case no precipitate forms after a few days, the culture is considered negative as far as micro-organisms of the enteric group are concerned.

If physicians are supplied with sterile swab-tubes (diphtheria swabs) for blood collection, the laboratory work is relatively simplified and may be limited to transfer of swabs into medium tubes, watching the culture daily and plating only those suspected of showing growth. In this way routine repeated plating, which is often associated with some degree of contamination, is eliminated.

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* Received for publication, August 20, 1947.

THE QUANTITATIVE DETERMINATION OF PULMONARY HEMORRHAGE*

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In the course of our studies on the production of pulmonary hemorrhage in cats by large decelerative forces, the need for an accurate description of interstitial hemorrhage was recognized. After various methods were tried and found unsatisfactory, the present procedure was developed. This procedure is based on a chemical determination of residual blood present in the lungs of cats which have been simultaneously bled and perfused. In addition to the fact that this method permits a quantitative expression of interstitial hemorrhage, the further advantages of objectivity, simplicity and consistent accuracy are deserving of note.

METHOD

After anesthetization with nembutal the cat is placed supine on an animal board. A transverse incision is then made across the epigastrium and continued upward in the form of a longitudinal incision through the thoracic cage at either anterior axillary line as far as the clavicles. An incision through the diaphragm separates it from its anterior attachment, and the anterior thoracic flap is reflected cephalad thus exposing the heart and great vessels and a major portion of both lungs. Immediately after opening the thorax, 0.5 cc. of heparin is injected into the right ventricle, and after about thirty seconds enough blood is withdrawn for a hemoglobin determination.¹ Following the withdrawal of blood a 15-gauge needle, through which the perfusion fluid is delivered, is introduced into the right ventricle, and another 15-gauge needle, through which the drainage of the pulmonary circulation passes into a receptacle, is introduced into the left ventricle. The perfusion fluid (saline) passes through the pulmonary circulation as soon as the clamp on the tubing is released, and blanching of the lungs is noted within a few seconds. After the perfusion has started, the superior and inferior vena cavae are clamped with hemostats (Fig. 1). The perfusion is continued until the fluid leaving the left atrium has been perfectly clear for at least one minute. This ordinarily takes from five to ten minutes in the uninjured lungs and requires about 500 cc. of saline. In hemorrhagic lungs the perfusion time is frequently extended to ten or fifteen minutes, and the amount of saline may be increased to 1000 cc. The rate of flow of the perfusion fluid is regulated to prevent excessive pulmonary edema, especially during the first few minutes of the perfusion when the greater portion of the pulmonary intravascular blood is removed.

* Received for publication, June 13, 1947.

† With the technical assistance of Mr. G. B. Ellis, Mr. L. D. Case, Sr., and Miss J. Masterson

After the perfusion is completed, the lungs are removed from the thoracic cavity and the individual lobes are placed in weighed Petri dishes. The pulmonary lobes are then dried to constant weight in an oven at 68 C. for a minimum of twenty-four hours. The dishes are re-weighed and the weights of the individual lobes are obtained by difference. Each lobe is pulverized in a mortar, acidified, extracted with a 1:4 mixture of 95 per cent ethyl alcohol and ethyl ether, and its original hemoglobin is converted to pyridine ferrohemochromogen



FIG. 1. Method of perfusion. A, inflow needle; B, outflow needle; C, hemostat on S.V.C.; D, hemostat on I.V.C.; E, area of hemorrhage.

according to the method of Flink and Watson.²

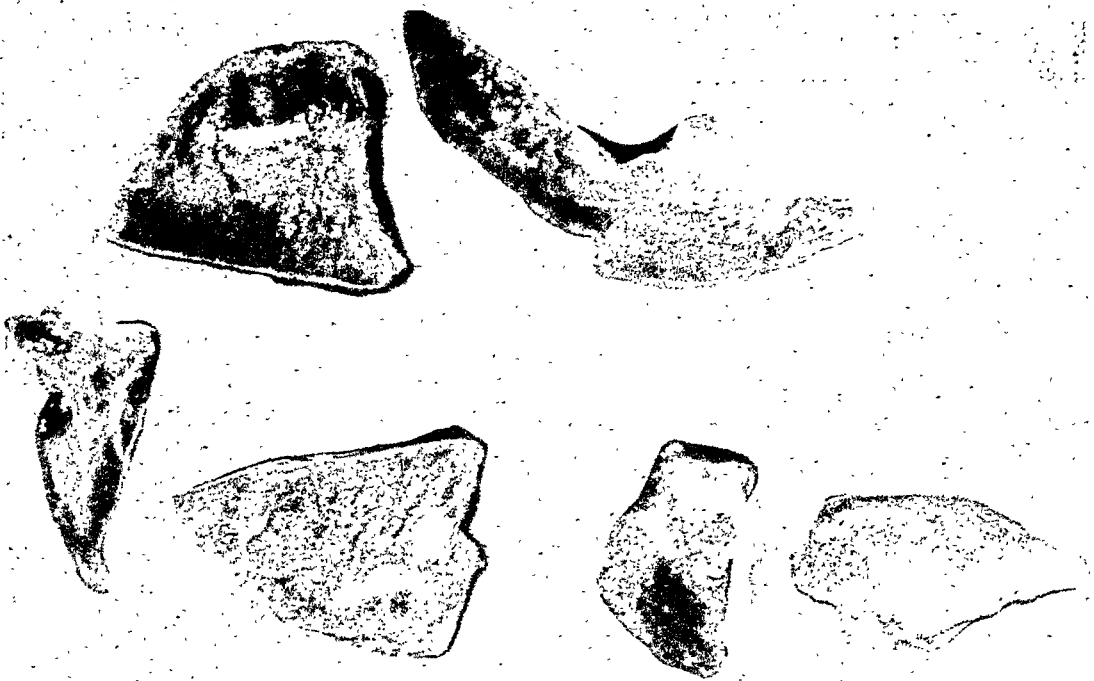
RESULTS

In order to determine the reliability of this procedure, a series of normal values was obtained by the extraction of 62 lobes from eleven fasting, afebrile cats. The results are shown in Table 1. Figure 2 illustrates the typical appearance of normal cat lungs following perfusion.

The term "equivalent amount of blood" seems especially useful in our problem of determining hemorrhage and is derived in the following manner: (1) Number of cc. of extravasated blood per lobe =
$$\frac{\text{mg. hemoglobin recovered from lung lobe}}{\text{mg. hemoglobin per cc. of blood}};$$



2



3

FIG. 2. Normal Lung After Perfusion.
FIG. 3. Hemorrhagic Lung After Perfusion.

$$(2) \text{ Number of cc. of blood per gm. of dry lung tissue} \\ = \frac{\text{equivalent amount of blood per lobe}}{\text{dry weight of lung lobe in gm.}}$$

To determine the actual recovery of the blood present in the lungs, known amounts of hemoglobin were injected into five lung lobes. An average recovery of 96.6 per cent (range from 89.7 to 103 per cent) was obtained.

Areas of pulmonary hemorrhage produced in cats subjected to large decelerative forces remained unchanged, while blanching of the uninjured tissue was evident from the onset of perfusion. Analysis of 25 hemorrhagic lung lobes from these cats gave an average equivalent amount of blood per lobe of 0.84 cc. (1.62 cc. per gram of dry lung tissue).

Figure 3 illustrates hemorrhage well delineated in perfused lungs.

TABLE 1
SUMMARY OF FINDINGS IN NORMAL LUNGS OF 11 CATS

LOBE	NUMBER OF LOBES	AVERAGE DRY WEIGHT IN GM.	AVERAGE TOTAL HEMOGLOBIN IN MG.	AVERAGE EQUIVALENT AMOUNT OF BLOOD		
				CC. PER LOBE	DRY LUNG TISSUE, CC. PER GM.	STANDARD DEVIATION
Right upper.....	10	.39	.61	.0053	.014	.010
Right middle.....	10	.24	.39	.0039	.016	.014
Right lower.....	11	.85	.71	.0066	.008	.004
Accessory.....	11	.24	.40	.0045	.018	.015
Left upper middle.....	10	.50	.74	.0062	.013	.010
Left lower	10	.89	.80	.0056	.009	.008
Averages for all 62 lobes.....		.52	.60	.0054	.013	

DISCUSSION

A quantitative method for determining interstitial hemorrhage is a desideratum which needs no special emphasis. The procedure outlined, although limited to the lungs by the nature of our problem, has a universal application. This may be in the form either of localized perfusion of certain anatomic regions or organs or as total body perfusions when measurements of several coexisting hemorrhages (*e.g.*, brain, lungs and kidneys) are desired.

Although this method has been employed in quantitation of hemorrhage only in cats, it is, of course, applicable to other laboratory animals.

CONCLUSION

Our experience has shown that the method of quantitatively determining pulmonary hemorrhage which has been described consistently gives results that are reliable and meaningful expressions of interstitial hemorrhage.

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ISOLATION OF PARTICLES FROM ASPIRATED STERNAL MARROW FOR BIOPSY*

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Hematologists, pathologists and experienced medical technologists are well aware that in some instances particles of marrow are difficult to isolate from the blood of the aspirated marrow specimen, particularly when these anatomic-physiologic structures are very small and few in number. It is further agreed among students of the bone marrow organ that a careful cytologic examination of smear and imprint preparations^{3, 5} does not yield conclusive evidence as to the presence or absence of structures such as those that characterize tuberculosis, sarcoidosis, brucellosis, early primary and metastatic malignant neoplasms or pathologic states of the vascular system. Thus, the importance of making histologic sections from sternal marrow particles is at once apparent. For the successful demonstration, however, of the above cited disease processes, which may or may not be widely scattered, it is imperative that from 2 to 5 cc. of marrow substance be aspirated to make certain that a representative specimen of the sternal bone marrow is furnished for biopsy. It is further emphasized that aspirated marrow must be placed immediately in a properly prepared glass vial, that is, the inside of the vial should be coated with a thin film of paraffin and a small amount of powdered heparin‡ should be thoroughly distributed over the well hardened and dry paraffin surface before the marrow is placed in the vial. Instantaneous mixing of the anticoagulant and the marrow is encouraged by gently inverting the vial several times. The amount of heparin necessary for preventing clotting of 1 cc. of marrow varies from batch to batch, although the quantity that covers the broad end of a toothpick is in general adequate.

It is the purpose of this communication to present a method that: (1) permits rapid preparation of marrow particles from the aspirated marrow regardless of their size; (2) assures minimum loss of the particles thus increasing the chances of detecting scattered lesions; and (3) prevents cytolysis and production of artefacts by the rapid and uniform fixation of the marrow particles.

MATERIALS

The following materials are needed:

An illumination box with a frosted glass plate top and a 60 watt 120 volt light bulb (Fig. 1);

Four Petri dishes each with a diameter of about 9.0 cm.;

* Received for publication, June 26, 1947.

† Parke, Davis and Company Research Fellow in Hematology.

‡ Dog Liver heparin lot 152, or any lot made according to the formula of lot 152, made by Hynson, Westcott and Dunning, Baltimore, Maryland.

One flat bottomed glass receptacle about 5.0 cm. in diameter, the inside of which is coated with a thin film of paraffin;

A medicine dropper and a few wooden applicators.

METHOD

After 1 cc. of the heparinized marrow specimen has been removed for concentration purposes,^{1, 2, 4} the remaining amount is poured in the paraffin coated glass receptacle. The latter is then slightly tilted to encourage visualization of particles for the purpose of making imprints.⁵ Particles of marrow range in appearance from translucent to opaque and vary in color from light gray to deep red.

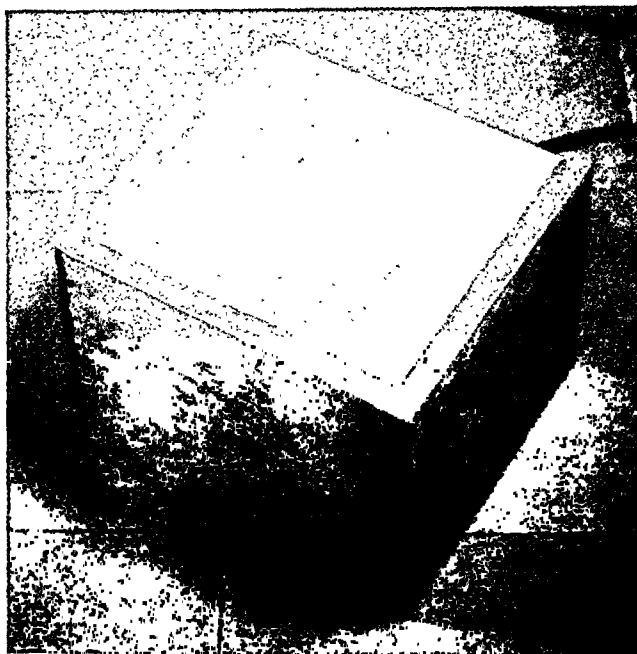


FIG. 1. Illumination box with frosted glass top. The back of the box is left open to prevent heating of the glass cover and of the fixing fluid.

The particles may be difficult to see, particularly when they are very small, that is, less than 0.5 mm. in diameter, and float in the blood of the specimen. It has been pointed out elsewhere that normally particles range in size from 0.5 to 1.0 mm.⁴ but may be as large as 6.0 mm. and as small as 0.2 mm. in certain pathologic states of the marrow. To bring about a better visualization of these particles, a small amount of physiologic sodium chloride solution may be added to the marrow specimen if imprints are to be made. The preceding step should be carried out as quickly as possible to prevent "soaking" of the particles, thus encouraging deterioration and the production of artefacts. After several particles have been imprinted, the marrow specimen is washed into one of the Petri dishes with freshly prepared fixing fluid (neutral formaldehyde 40 per cent saturation, 1 part, physiologic sodium chloride solution 9 parts). The dish is then placed on the glass top of the illumination box (Fig. 1) and checked for visualization of

the marrow particles. Generally, large particles will be readily seen (Fig. 2A), although the mixture may be too opaque for detecting very small ones. About one-half of the mixture is then poured into another dish and equal amounts of fixing fluid are added to both specimens. At this point, small particles are visible (Fig. 2B). The particles of marrow are transferred with a medicine dropper to another glass dish containing fixing fluid (Fig. 2C). If the fluid becomes turbid

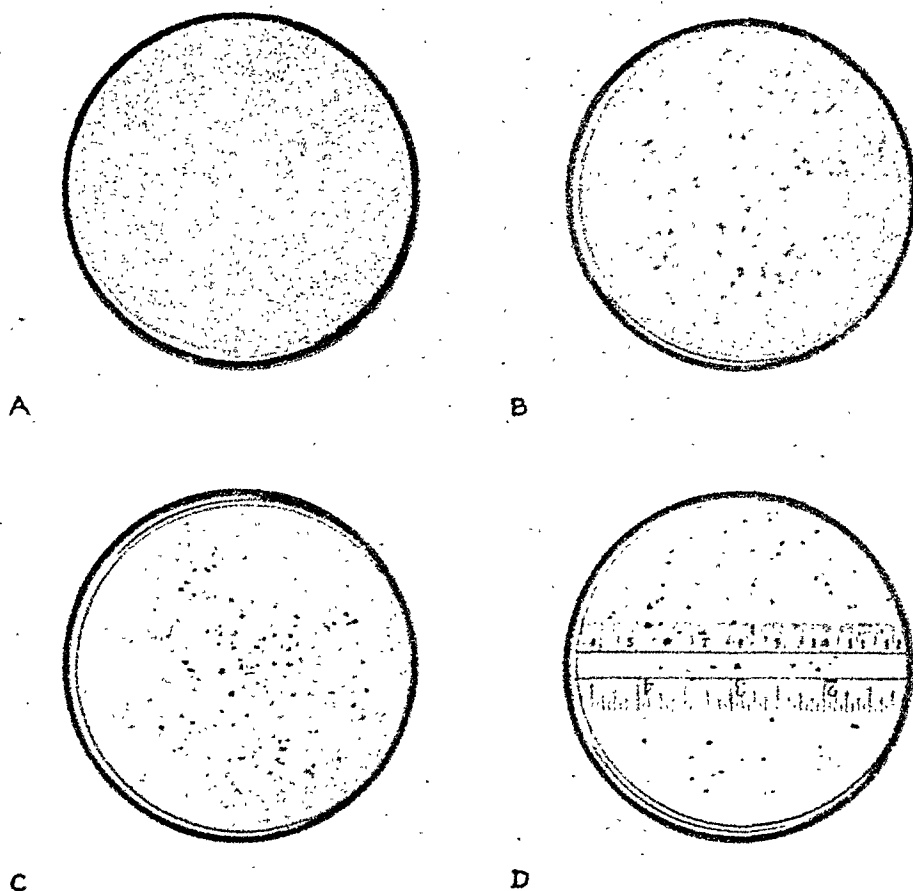


FIG. 2. A, Petri dish containing marrow specimen diluted with formaldehyde-saline fixing fluid. Large particles of marrow are easily visible. The particles are represented in the photograph as opaque irregular-sized structures. B, Petri dish with one-half of specimen "A" diluted with formaldehyde-saline fixing fluid. Large particles can be seen clearly, but particles of less than 1.0 mm. are just visible. C, Petri dish containing particles of marrow from "A" and "B". Observe the conspicuous size range of the particles. The formaldehyde-saline fixing fluid is slightly cloudy from transferred blood. D, Petri dish with particles removed from "C" for final fixation. Because the formaldehyde-saline fixing fluid is clear, range of size and number of the particles can be determined. The transparent ruler is under the glass receptacle.

from transferred blood, it is best to remove the marrow particles and place them in another receptacle containing clean fixing fluid. The particles should remain in fixing fluid for several hours or over-night. While undergoing complete fixation, the range of the diameter of the particles may be determined by transferring them directly on to a transparent ruler, as shown in Fig. 2D, and the

number by placing a square subdivided into smaller squares (drawn with India ink on a clean x-ray film) under the glass receptacle. Properly fixed particles can be shipped to distant laboratories for histologic examination. It is important to place enough fixing fluid in the shipping container to prevent drying of the marrow particles. Sections are made according to methods published elsewhere.^{6, 7, 8}

SUMMARY

A method is described for rapid preparation of aspirated particles of bone marrow for biopsy. The procedure employs an illumination box with a frosted glass top large enough to hold several glass receptacles. The marrow particles are visualized by diluting the aspirated specimen with fixing fluid. The method assures maximum yield of particles for histologic work and thus widely scattered lesions of the bone marrow organ may be more readily detected.

Acknowledgment. The photographs were made by the Department of Medical Photography, Minneapolis General Hospital, Minneapolis, Minnesota.

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A METHOD FOR CROSS INDEXING PATHOLOGIC DIAGNOSES*

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Few laboratories can afford an elaborate system of indexing diagnoses of surgical and autopsy tissues. Most pathologists have to be satisfied with a numerical and a name file which permits the diagnosis for any particular patient to be found fairly readily, but does not lend itself to a general review of particular lesions or diseases. The following system is not original but has proved so satisfactory that it is deemed worthy of publication.

ANATOMIC SOURCE:	PATHOLOGIC DIAGNOSIS					
	Adenomatous Hyperplasia	Chronic Prostatitis	Occult Carcinoma	Adenocarcinoma, Scirrhous	Adenocarcinoma, Medullary	Leiomyoma
Prostate gland	S47-10	S47-10	S47-10	S47-61	S47-84	S47-26
		S47-26	S47-95	S47-65		S47-82
		S47-69				

FIG. 1. An index sheet for diagnosis of lesions of prostate gland. The accession numbers are listed below their respective pathologic diagnoses.

METHOD

When a tissue specimen is received, a record of it is entered in a daily register and it is given an accession number. On the following day, after examination of the sections, the lesions are coded according to the anatomic source and the pathologic diagnosis. For example, sections of a prostate gland may show occult carcinoma, adenomatous hyperplasia and chronic prostatitis. The "index summary" will read, accordingly:

Anatomic source: Prostate gland
 Diagnosis: Occult carcinoma
 Adenomatous hyperplasia
 Chronic prostatitis

When the report is typed, the diagnosis is entered in a combined anatomic and diagnostic loose leaf ledger, as indicated in Figure 1. A single sheet suffices for coding twelve or more diagnoses for each organ or tissue, leaving an adequate margin for binding. Many cases can be listed on each sheet, although diagnoses of organs which are removed frequently may need two or three sheets in the course of a year. The pages are inserted alphabetically according to the organ or tissue involved. When the ledger is filled, it is bound in book form for permanent reference. Each book is dated and numbered consecutively. Any dis-

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ease entity can be reviewed conveniently and quickly, since it will be coded and indexed by name.

This system has been very valuable in our training program for residents preparing for their specialty boards and is equally adaptable to the needs of colleagues wishing to present material at medical meetings. It takes only a little extra time to set up and maintain such a file and it requires little additional space. A similar system is used for coding diagnoses on tissues removed post mortem.

It is well to remember that only important changes should be listed, as no useful purpose is served by listing minor lesions, such as cloudy swelling or slight congestion.

SUMMARY

A method is described for indexing pathologic diagnoses of tissues removed post mortem or at operation.

TECHNICAL SUGGESTIONS

A HELPFUL TECHNIC IN MAKING ACID-FAST STAINS

The following suggestion is offered for steaming when making acid-fast stains. The method was shown to me by Dr. Steinar Hauge of the Department of Bacteriology, College of Veterinary Medicine, Oslo, Norway.

After the smear has been fixed, a piece of medium or heavy filter paper, measuring about $3\frac{1}{2}$ inches by $2\frac{1}{4}$ inches is folded over the slide (Fig. 1) so as to cover the bottom and top surfaces. The purpose of the filter paper is to absorb the stain

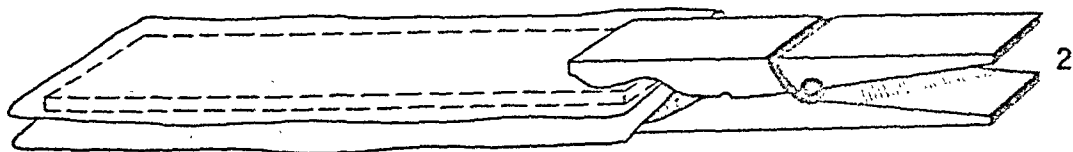
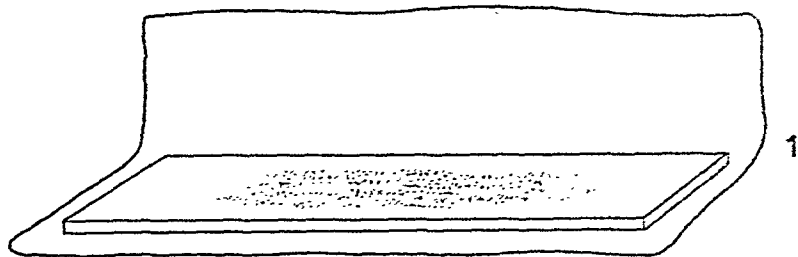


FIG. 1. A 3 x 1 inch slide containing material to be stained is placed on a piece of heavy filter paper, approximately $3\frac{1}{2} \times 2\frac{1}{4}$ inches in size.

FIG. 2. Fold filter paper over slide as shown, then grasp slide at one end by a spring type clothespin. The stain can then be applied and the slide steamed over a Bunsen burner.

and prevent drying. A spring-wire type of clothespin is used to secure the paper to the slide and serves to hold the slide conveniently at one end (Fig. 2) during the steaming process. The stain is then dropped onto the filter paper. After steaming begins, more stain may be dropped onto the paper to insure that the paper does not curl but will remain in apposition with the surface of the slide.

I have found this method advantageous because of the ability to obtain a more uniform stain with it. Precipitation of the stain is eliminated and considerably less stain is required. Where the application of heat is necessary this method probably can be employed with success in other staining procedures.

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METHODS OF PREPARING STERILE DRY SYRINGES

The following methods of preparing sterile syringes, although not original, are presented because they are superior to boiling and fishing syringes and needles out of water baths, and are also superior to wrapping them in cloth. The unit can be assembled prior to sterilization and can be examined before use without contaminating the set.

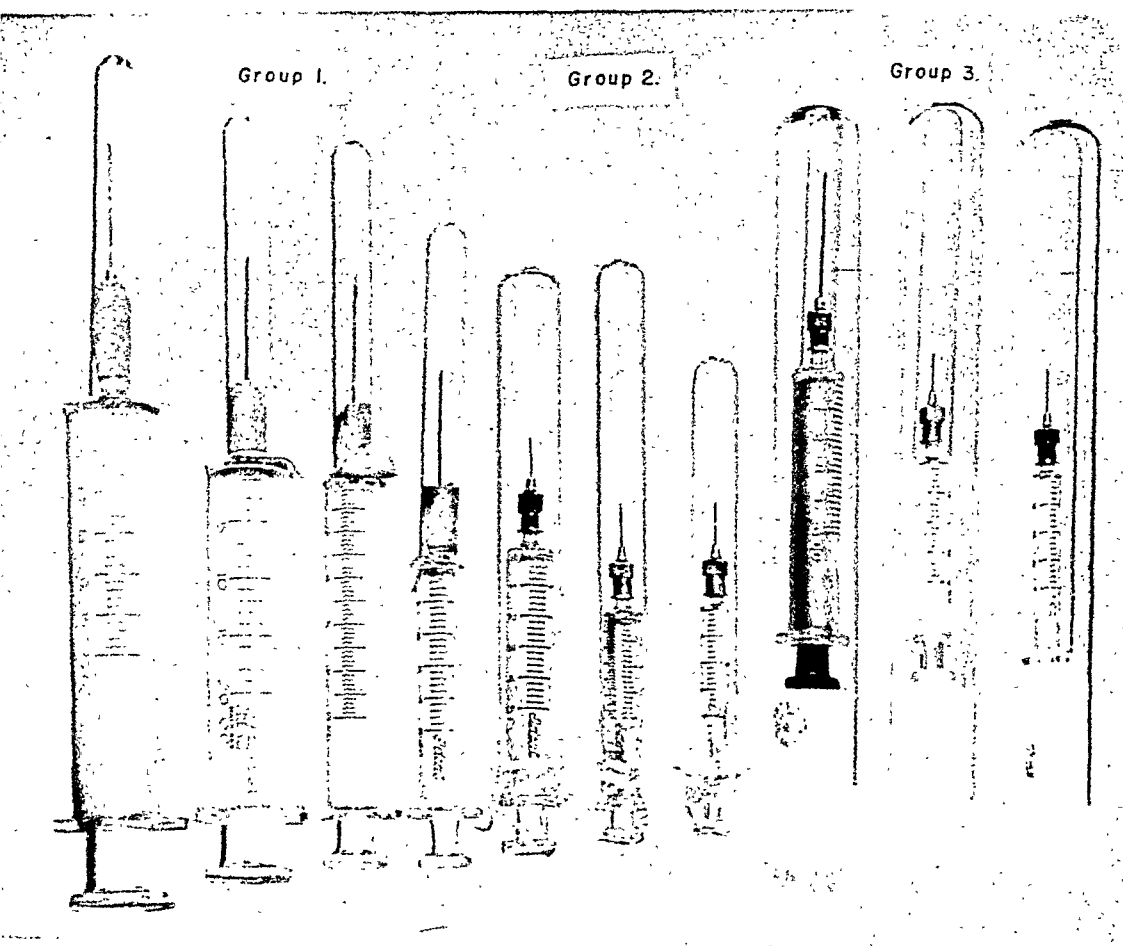


FIG. 1

The first method (Fig. 1, Group 1) is adaptable to any size syringe or needle. The needle is placed on the tip of the syringe; gauze tape is wound about the tip of the syringe and hub of the needle so that a test tube may be fixed snugly over the needle to serve as a needle protector. A short piece of gauze tape extends beyond the mouth of the needle protector so that it can be removed without loosening the needle. The whole set is sterilized and may be kept sterile until ready for use.

A second and better method is to cover the assembled needle and syringe with a test tube whose bore will not extend beyond the mouth end of the syringe. The plunger is fixed in position by wrapping it and the mouth of the tube with cellophane and tying the cellophane, as shown in Figure 1, Group 2.

In the third method (Fig. 1, Group 3) a small test tube is used to cover the needle and the entire syringe and needle is placed in a larger tube and held in place by a cotton plug. Tubes large enough to hold a 10 cc. syringe and attached needle may be purchased from supply houses.

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CLEANING USED SLIDES AND COVER GLASSES

The following suggestions may be of value to laboratory workers in cleaning batches of used slides and cover glasses. Separate slides and cover glasses into the following groups: (1) unmounted slides having no immersion oil or embedding material on them, (2) unmounted slides with immersion oil, (3) unmounted slides with paraffin sections, (4) mounted slides with paper labels, (5) all other mounted slides.

EQUIPMENT AND SUPPLIES

- 1 pair pointed forceps for handling slides and cover glasses
- 1 long-handled razor with disposable blades, or scalpel
- 1 small tripod or ring stand
- 1 wire gauze square with asbestos center
- 1 burner
- 1 pyrex beaker, 250 cc.
- 2 pyrex beakers, 400 cc.
- 3 Coplin jars
- used and new xylol
- potassium bichromate-sulfuric acid cleaning solution (containing no water)
- clean alcohol, 70 to 95 per cent
- 1 clean heavy wiping cloth
- 2 clean, soft, white, lintless wiping cloths

PROCEDURE

1. *Removal of paper labels.* Place slides label-end down in a beaker or tumbler containing just sufficient warm water to cover the labels. As soon as the labels become loose, remove the slides and wipe them dry. Paper labels covered with cellulose tape or shellac should be removed with a razor blade.

2. *Removal of cover glasses from mounted specimens.* Warm mounted slides, cover glass side up, over a low flame. With a razor or an old scalpel push the cover glass off into a wide-mouthed bottle containing used xylol. Some slides may have to remain immersed in xylol for several hours to loosen the mount. This method of removing cover glasses is preferable to dipping in boiling alkali, which may cause the surfaces of the slides, cover glasses and beakers to become etched.

3. *Removal of immersion oil, mounting mediums, paraffin and other embedding material.* Place the slides from which the cover glasses have just been removed, on end in a storage jar containing used xylol. Slides stuck together should be separated with a razor blade before being placed in xylol. The slides should be covered by xylol and should be kept in this solvent at least over-night. The most satisfactory containers for this purpose are straight-walled, covered, wide-mouthed glass specimen jars just deep enough to accommodate the ordinary 3 inch by 1 inch slides set on end.

4. *Removal of stained or unstained smears and sections.* Slides free of immersion oil and mounting or embedding mediums may be quickly freed of most of the remaining superposed material with a few strokes of the razor blade.

5. *Final cleaning procedure.* Set up an "assembly line" as follows:

- a. Coplin jar of used xylol;
- b. Coplin jar of new xylol;
- c. Place a 250 cc. pyrex beaker about two-thirds full of strong cleaning solution on an asbestos wire gauze over a low flame;
- d. In front of the beaker containing cleaning solution, place two 400 cc. pyrex beakers containing hot water;
- e. Coplin jar containing 70 to 95 per cent clean alcohol.

6. Place from ten to fifteen used slides in the jar of used xylol. With the forceps, remove one slide at a time from the used xylol, drain it on the side of the jar, swish it back and forth in the new xylol, drain as before, wipe with the heavy cloth, taking care to keep both hands covered with the cloth to avoid cutting the hands. Repeat until all the slides are out of the first jar, then insert another batch of from ten to fifteen slides in the jar of used xylol and clean as before.

7. Immerse one of the wiped slides completely in the hot cleaning solution, then remove it with the forceps, drain it well against the side of the beaker, simultaneously inserting another slide in the hot solution with the other hand. To avoid excessive corrosion of the forceps, quickly plunge the cleaned slide into the first beaker of hot water. Swish it in the water a few times, drain it against the side of the beaker, then swish it in the second beaker of hot water, continuing to handle with the forceps only. Drain and wipe the slide with a clean, lintless cloth. Swish the slide in alcohol and wipe it dry with another clean, lintless cloth. As each succeeding slide is removed from the hot cleaning solution, another slide, wiped dry after treatment with xylol, is dropped into the hot cleaning solution. Cover glasses are treated like slides, except that they are not dropped into the hot solution, but are carefully dipped several times in the hot acid-bichromate until free of extraneous matter before rinsing in hot water.

The cleaning solutions should be kept hot but it is not necessary for the solution to reach the boiling point. The hot water in the rinsing beakers should be changed for every two batches of slides. Occasionally, more cleaning solution will have to be added to the beaker. If the cleaning process has been properly carried out, a drop of distilled water will spread evenly on the cleaned glass surface.

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LYMPHOCYTIC INFILTRATION AND HEMOPOIESIS IN THE LIVER OF TUMOR-BEARING HAMSTERS*

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Aggregates of lymphocytes and plasma cells occur in the periportal connective tissue in the livers of Syrian hamsters implanted with a rapidly growing, mixed-cell sarcoma, but similar aggregates are absent in the livers of normal hamsters and are not observed in livers of several other laboratory rodents. The purpose of this investigation is to determine the relation and significance of this infiltration to the development of the implanted tumor.

Lymphocytes and plasma cells, although usually stressed in text-book descriptions of cirrhosis of the liver, are specifically mentioned by only a few of the many investigators interested in hepatic cirrhosis. Even¹¹ stated that the first response in experimental cirrhosis in rabbits is marked by dilation of the capillaries and infiltration of round cells. Infiltration of periportal areas by round cells has also been reported to accompany proliferating connective tissue in the livers of patients suffering from malarial cachexia (Craig⁵).

Several authors who have been interested in the effects of chemical carcinogens on the liver have mentioned the presence of periportal lymphocytes in relation to the histogenesis of experimentally induced hepatic and extrahepatic tumors. Lymphocytes infiltrated the proliferating fibrous connective tissue of the liver in rats at the end of two months of feeding butter-yellow (Sugiura and Rhoads²²). After injecting dibenzanthracene into rats, Goerner and Goerner¹⁴ observed infiltration of small round cells into necrotic areas about the central vein and in the periphery of the hepatic lobules. Slight periportal infiltration of lymphocytes and plasma cells was present in the livers of rats injected with dibenzanthracene (Cornil, Paillas and Castueil³) and in the livers of hamsters injected with benzpyrene (Halberstaedter¹⁶). Fibrosis, aggregates of lymphocytes, and persistence of bile ducts followed the stage of active inflammation in an hepatic sarcoma that was produced by methylcholanthrene in rats (Eisen¹⁰). Kahlstorf¹⁵ reviewed the extensive literature on infiltration in the liver, beginning with 1860, and stated that he observed periportal infiltration in 20 cases of carcinoma of the stomach and intestine in man.

Conditions that obstruct the blood and lymph courses are often followed by infiltration by the cells that are present in the blood. In chronic lymphoid leukemia lymphocytes may accumulate within blood sinusoids and vessels of the liver (Green,¹⁵ MacCallum,²² Mallory²²). Similar accumulations have been described in leukemia of the dog (Bloom and Meyer¹) and in experimentally

* This study was aided by grants from the Bauer and Black Division of the Kendall Company and from the Research Council of the Graduate School of the University of Colorado. Part of the work was done while the senior author was a recipient of a Finney-Howell Medical Research Fellowship. Received for publication, August 15, 1947.

induced leukemias of mice (Law²¹) and chickens (Burmester, Prickett and Belding² and Olson²³).

Lymphocytes and plasma cells, normally present in bone marrow, have also been found associated with extramedullary myelopoiesis in the liver and other organs (Jordan^{17a}). Numerous clinical reports show that extramedullary myelopoiesis is associated with various pathologic conditions in man, such as leuko-erythroblastic anemia (Mendeloff and Rosenthal²⁶), erythroblastosis (Ferguson¹²), and in experimental animals following injection of saponin (Wuyts³⁷) and vaccine of *E. coli* (Lang³⁰). The relation of the presence of tumors to extramedullary hemopoiesis in the liver, however, has been considered by only a few writers. Parsons²⁹ noticed infiltration of round cells and the presence of myelocytes and immature leukocytes in the portal areas in the livers of mice having leukemia coincident with a mixed-cell sarcoma. Takizawa³³ found myeloid foci in the livers of cats having primary hepatomas produced by feeding o-amino-azotoluene.

TABLE 1

RELATION OF INANITION TO PERIportal INFILTRATION OF LYMPHOCYTES AND PLASMA CELLS AND NECROSIS IN THE LIVERS OF 8 MALE HAMSTERS

ANIMAL NUMBER	INITIAL WEIGHT GRAMS	WEIGHT LOST BY INANITION		DURATION OF INANITION IN DAYS	AGE WHEN KILLED, IN DAYS	INFILTRATION	NECROSIS PRESENT
		Grams	Percentage				
334	89.2	27.2	(30.5)	17	233	0	+
335	87.0	32.5	(33.5)	17	224	0	0
336	96.0	33.0	(34.4)	17	53	0	0
338	107.5	36.5	(33.8)	17	53	0	0
343	76.5	35.4	(46.3)	24	86	0	0
344	82.5	28.0	(34.0)	24	86	0	0
345	74.0	26.0	(31.1)	24	86	0	0
346	97.0	31.5	(32.5)	24	86	0	0
Mean.....	89.96	31.2	(34.4)	20.5	113.5		

Tchakhotine³¹ suggested that protein substances from tumors initiate development of hemopoietic tissue.

MATERIALS AND METHODS

The animals used in this work comprised 70 tumor-bearing hamsters (none of which had a definite metastasis to the liver), 8 starved hamsters and 20 control hamsters, all *Mesocricetus auratus*. The animals were from a closely inbred stock and included both sexes and various ages. In addition, the normal livers of 1 young guinea pig and 3 adult guinea pigs, 1 adult wild muskrat (killed in November 1946), and 2 adult white rats were examined for comparison.

The tumor-bearing hamsters had been subpannicularly implanted with various transplant generations of a mixed-cell sarcoma that was originally induced by injections of 9,10-dimethyl-1,2-benzanthracene (Crabb,⁵ and Fig. 1, animal A4:1F). In order to determine whether the periportal infiltration was due

directly to the effects of the sarcoma or was related to the resulting cachexia, two groups of 5 normal hamsters were kept on a restricted stock diet until they were reduced to a state of extreme inanition as evidenced by death of one animal in each group. The percentages of weight lost in the 8 surviving hamsters are given in Table 1.

The tumor-bearing animals are considered in 5 major groups (Table 2) based on the relative duration (number of days from implantation to death of the host) of the implanted sarcoma, a method which serves as an index of the growth and toxicity of necrotic changes in the tumor. The duration was greatly increased and the effects of the implanted sarcoma thus prolonged by subtotal removal of the tumor once or twice before the host became cachectic.

Thick pieces of both right and left anterior lobes of the liver were routinely fixed in sublimate-alcohol solution, imbedded in paraffin, sectioned at 8 microns and stained with Grubler's toluidine blue or by the Feulgen method (Cowdry²). Staining with toluidine blue proved to be a speedy, efficient method for identi-

TABLE 2

INCIDENCE OF PERIPORTAL LYMPHOCYTIC AND PLASMA CELL INFILTRATION, HEMOPOIESIS AND LYMPHOID NODULES IN THE LIVER

GROUP	NUMBER OF ANIMALS	INFILTRATION	HEMOPOIESIS	LYMPHOID NODULES	CIRRHOSIS	NECROSIS
Control.....	20	0	0	0	0	0
Inanition.....	8	0	0	0	0	1
Tumor						
1-5 days.....	9	9	0	0	0	0
11-20 days.....	9	9	2	1	1	0
22-30 days.....	17	17	8	2	0	0
31-91 days.....	17	17	13	6	1	1
Subtotal.....	18	18	11	5	1	2
Total tumor.....	70	70	34	14	3	3

fication of plasma cells and possessed other advantages over hematoxylin and eosin. Delafield's and modified Heidenhain's iron-hematoxylin, Mallory's phosphotungstic acid-hematein-ammonium, and Mallory's aniline blue stain for white fibrous connective tissue were also used for photographic purposes or to check histologic structures in the liver.

OBSERVATIONS

The liver of the normal hamster is similar in structure to that of most mammals in that the fibroelastic connective tissue, which is a part of Glisson's capsule (Whitney³²) does not delimit the hepatic lobules, but is limited chiefly to areas surrounding the portal vessels and bile ducts. The periportal connective tissue and blood sinusoids in normal hamsters usually contain a few lymphocytes and occasional plasma cells and polymorphonuclear leukocytes, but these cells do not occur in aggregates as they do in the livers of the tumor-bearing hamsters.

A few mast cells occurred in the periportal tissue of the tumor-bearing hamsters

and were also found in this region in 2 of the 20 control animals. Mast cells, however, were found occasionally in the connective tissue around the gallbladder in most of the control and tumor-bearing hamsters.

Nuclei of parenchymal cells in the liver of tumor-bearing hamsters appeared to be similar in size and structure to nuclei in the liver of controls. A few binucleate cells occurred in the livers of both the normal and tumor-bearing hamsters, but no obvious differences were found in the number of binucleate cells in the two groups. In addition, no differences were observed in the size of hepatic nuclei in the two groups.

Cirrhosis and necrosis. The more widespread hepatic changes in the tumor-bearing animals were characterized by periportal infiltration and the occurrence of hemopoiesis and lymphoid nodules; however, cirrhosis and necrosis were found in the livers of a few tumor-bearing hamsters (Table 2). Portal cirrhosis (Fig. 20) occurred in three hamsters having tumors of varying durations. Lymphocytes and plasma cells were present in sinusoids (Fig. 21) and as isolated cells throughout the cirrhotic areas in these animals, but cells in these areas were not as numerous as in the areas of periportal lymphocytic infiltration (Figs. 14-16).

Focal necrosis occurred in three of the tumor-bearing hamsters. The size of the necrotic areas varied from a few degenerate cells (Figs. 17, 22) to larger areas having margins that showed beginning fibroblastic proliferation (Fig. 18).

Metastasis to the liver. No definite tumor growths were found in the livers of any of the 70 tumor-bearing hamsters. This absence is surprising in view of the fact that in man there is a remarkably high incidence of metastasis to the liver from primary neoplasms that are not situated within the areas drained by the hepatic portal system and that the liver is the site of blood-borne metastases in from 24 to 36 per cent of all classes of malignant tumors (Willis³⁶).

Periportal infiltration. Within twenty-four hours after the hamsters received implants of the tumor tissue, the number of individual lymphocytes and plasma cells increased, and aggregates of these cells formed within the lymphatics and sinusoids in the connective tissue surrounding the portal veins and bile ducts.

PLATE 1

Tissues represented by the figures in this plate were stained by the Feulgen method for thymonucleic acid. The nuclei of lymphocytes and plasma cells, represented by the round black dots, are darker than the nuclei of hepatic cells because they contain more thymonucleic acid. All figures are magnified 150 times.

Fig. 1. Slight infiltration of lymphocytes and plasma cells in the periportal connective tissue of a hamster having a mixed-cell sarcoma for fifteen days.

Fig. 2. Infiltration at the bifurcation of a portal vein in the liver of a hamster having a sarcoma for twenty-two days.

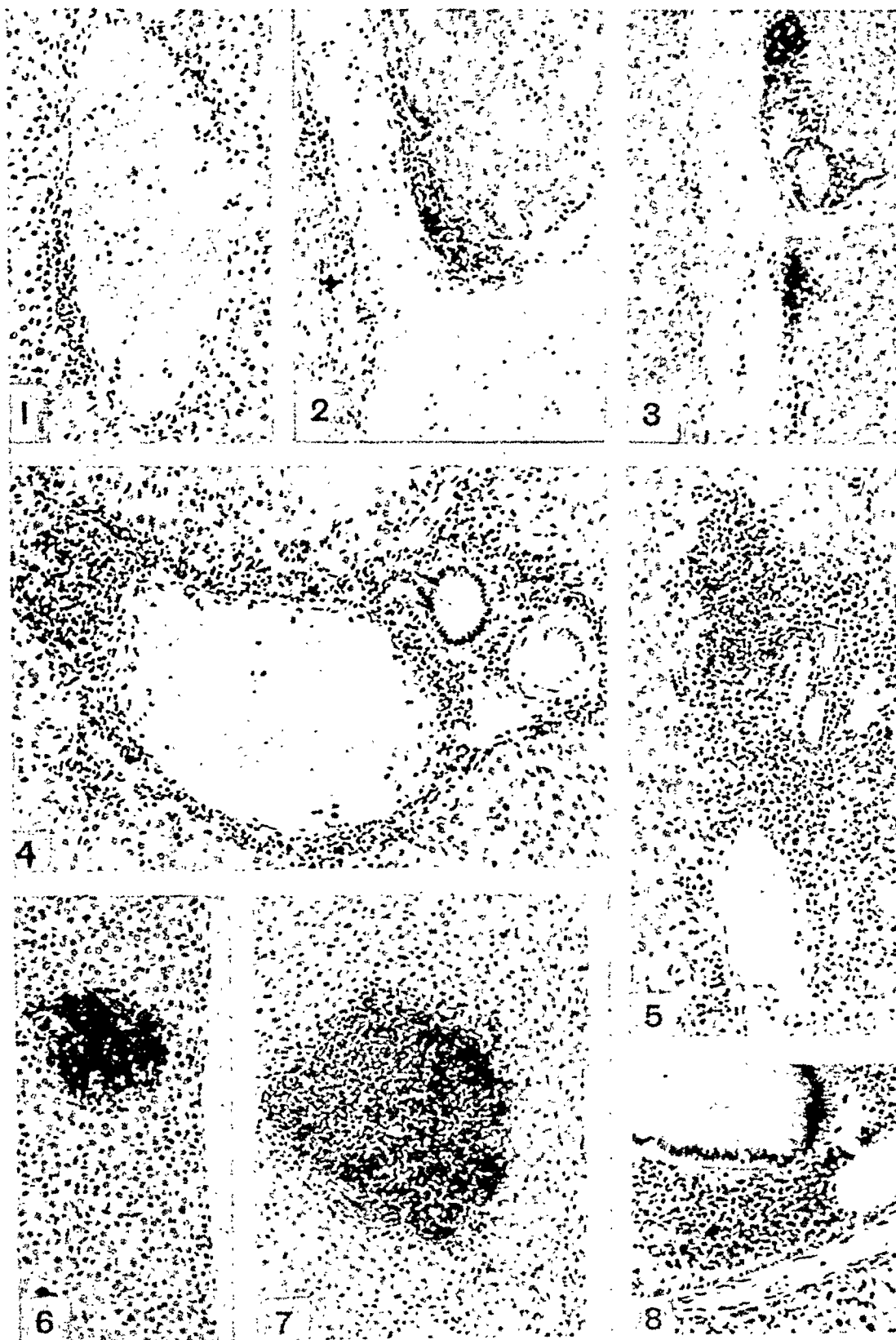
Fig. 3. Longitudinal section of a hepatic portal vein from the same animal as in Figure 2 to show lymphocytes and plasma cells in the periportal areas.

Figs. 4 and 5. Infiltration of portal vessels from a hamster having a tumor fifty-six days (Fig. 4) and from one having a tumor 158 days, a period during which two subtotal removals of the tumor were made (Fig. 5).

Figs. 6 and 7. Lymphoid nodules near the margin of the liver from a hamster having a tumor twenty-five days (Fig. 6) and from one having a tumor seventy-seven days (Fig. 7).

Fig. 8. Heavy infiltration in the connective tissue around a bile duct from the same animal as in Figure 5.

PLATE 1



The cells then continued to infiltrate this connective tissue until the lymphatics and sinusoids could no longer be distinguished (Fig. 10).

Variations in the amount of periportal infiltration during the first few days after implantation (Figs. 1, 10) suggest the possibility that the amount and condition of the tumor implanted is a factor responsible for the initial infiltration. Two hamsters that had been injected with massive doses of the necrotic fluid and fragments of living tumor tissue had livers that contained extensive infiltration of plasma cells and lymphocytes at the end of seventy-two hours (Fig. 12), while two other animals that had received similar doses of living fragments of the same tumor with only about one-fourth the amount of necrotic fluid had very small and comparatively few aggregates of these cells in the liver after the same period (Fig. 13).

Y-region of infiltration. Periportal infiltration of the connective tissue begins at the bifurcation, or y-region, of the vessels. The infiltration was noticeable fifteen days after implantation, became pronounced twenty-two days after injection of the tumor (Figs. 2, 3), and proceeded throughout the periportal areas until the infiltration extended about the bile ducts (Fig. 8) and about the larger blood vessels and bile ducts (Figs. 4, 5) as well as around the smaller terminal branches of these vessels.

The periportal areas, which are very narrow in normal hamsters (Fig. 9), were considerably widened before hemopoietic foci developed. The widening process is affected by plasma cells which encroach on the hepatic cells (Fig. 34) and appear to destroy them by secreting proteolytic enzymes which cause disintegration of the adjacent parenchyma (Figs. 34, 35). That the invading plasma cells secrete proteolytic enzymes is indicated by the fact that they were usually surrounded by vacuoles within the hepatic cells (Figs. 10, 11, 34). Similar vacuoles were present around plasma cells that had been ingested by megakaryocytes in the bone marrow (Fig. 28).

Russell's bodies. Fuchsinophilic inclusions in the cytoplasm of plasma cells (Russell's bodies of Mallory²³ and Stengel and Fox³¹) were found in the periportal infiltration in several tumor-bearing hamsters. Sections of the liver from 20 hamsters having tumors were stained with acid fuchsin, and the plasma cells were examined for fuchsinophilic inclusions. Russell's bodies were found in

PLATE 2

Tissues in all figures except Figure 9 were stained with iron-hematoxylin and eosin.

Fig. 9. Absence of periportal infiltration in a normal hamster. Feulgen stain. $\times 400$.

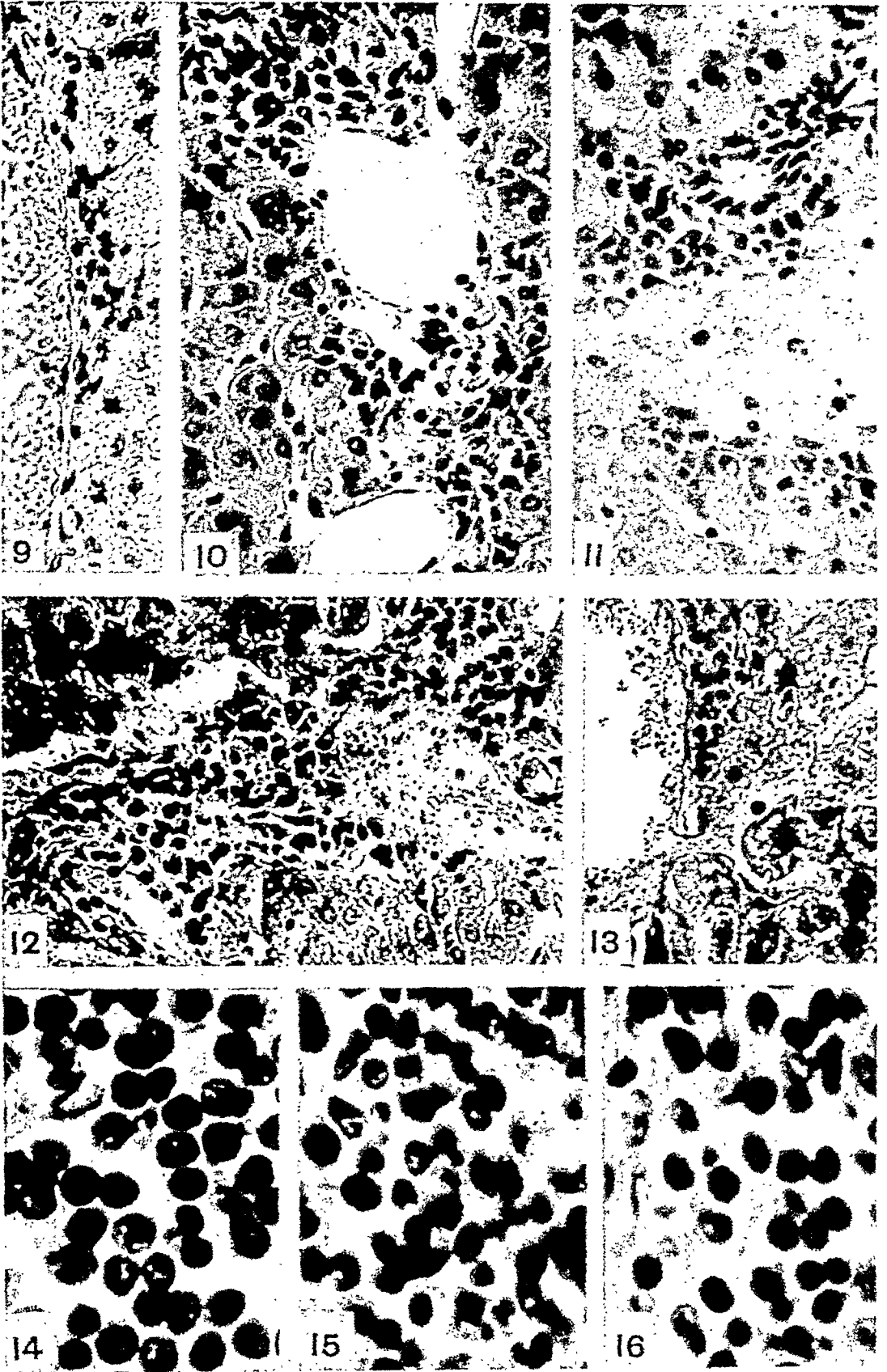
Figs. 10 and 11. Similarity in the periportal infiltration of lymphocytes and plasma cells in a hamster having a massive injection of necrotic tumor tissue for only forty-eight hours (Fig. 10) and in a hamster having a rapidly growing tumor for fifty-one days (Fig. 11). $\times 400$.

Fig. 12. Extensive infiltration of lymphocytes and plasma cells in the liver of a hamster that had received 1 cc. of a suspension of necrotic and minute bits of living tumor tissue seventy-two hours previously. $\times 400$.

Fig. 13. Area of greatest infiltration of lymphocytes in the liver of a hamster that had received implants of one-fourth the amount injected into the host in 12. The implantation (Fig. 13) was also made seventy-two hours previously. $\times 400$.

Figs. 14, 15 and 16. Sections showing the similarity in cells infiltrating the periportal connective tissue in the liver of hamsters having tumors twenty-three days (Fig. 14) fifty-one days (Fig. 15) and 158 days (Fig. 16). $\times 1200$.

PLATE 2



large numbers in the livers of 3 animals, but they were found only occasionally in the livers of the remaining 17 animals. In the 3 animals, the plasma cells that contained Russell's bodies were present in localized, extensively infiltrated areas as Dawson and Masur⁸ found in certain sinuses in the inguinal lymph nodes of normal rats. The livers of two of these hamsters contained hemopoietic areas, and in the third, lymphoid nodules were present.

Comparisons were made to determine if the Russell's bodies and erythrocytes of tumor-bearing hamsters stain similarly to these two elements in the rat (Kindred¹⁹). The Russell's bodies were definitely chromophobic, appearing as vacuoles, and the erythrocytes were likewise colorless in sections stained with toluidine blue or by the Feulgen method. Russell's bodies, however, were the same color as erythrocytes in sections stained with Mallory's aniline blue connective tissue stain, hematoxylin-eosin, and with a solution of less than 0.1 per cent acid fuchsin. These observations on the hamster support the tenet that Russell's bodies are hemoglobin (Jordan,¹⁷ Kindred¹⁹).

Cachexia. The periportal infiltration in the liver of tumor-bearing hamsters was considered as possibly being due to cachexia, a condition that commonly occurs in the presence of advanced tumors and in association with which hepatic cirrhosis has been known to develop (MacCallum²²). The livers of the eight starved animals, whose weight had been reduced 30 to 46 per cent (Table 1), were noticeably shrunken but did not contain periportal infiltrations. In the oldest animal of the group, there was a single necrotic area that contained pyknotic small and large lymphocytes and a few polymorphonuclear leukocytes (Table 1). With this exception, the number and structure of lymphocytes, polymorphonuclear leukocytes, mast cells and plasma cells were about the same in the starved as in the control animals. Numerous cytoplasmic vacuoles were characteristic of the hepatic cells in the starved animals (Fig. 24), but similar vacuoles were rarely found in the control and tumor-bearing animals (Fig. 25).

Hemopoiesis in the liver. Hemopoiesis occurred in areas of periportal infiltration in the liver of 34 of the 70 hamsters having tumors (Table 2). Extramedullary hemopoiesis was present in one animal as early as eighteen days after implanta-

PLATE 3

Tissues in all figures, except 23, were stained with iron-hematoxylin and eosin.

Fig. 17. Necrosis in the liver of a hamster having a mixed-cell sarcoma for thirty-five days. $\times 400$.

Fig. 18. Fibrosis surrounding a large necrotic area in the liver of the same animal as in Fig. 17. $\times 400$.

Fig. 19. Section of a necrotic area from the same hamster as in Fig. 17 to show plasma cells, lymphocytes, and dissociated parenchymal cells in a necrotic area. $\times 1200$.

Fig. 20. Cirrhosis in the liver of a hamster having a tumor for thirty-eight days. $\times 400$.

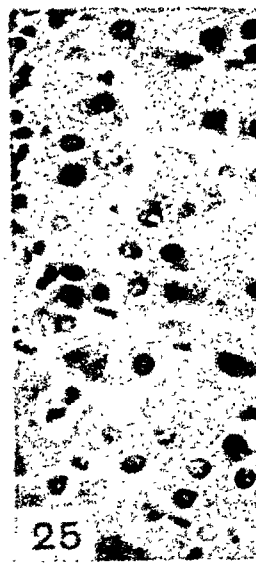
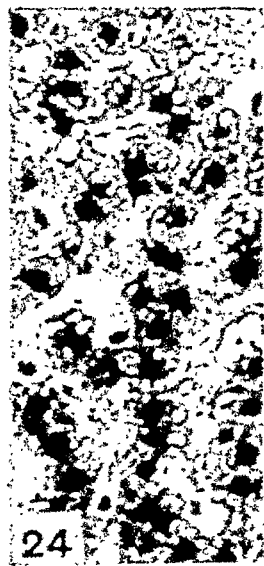
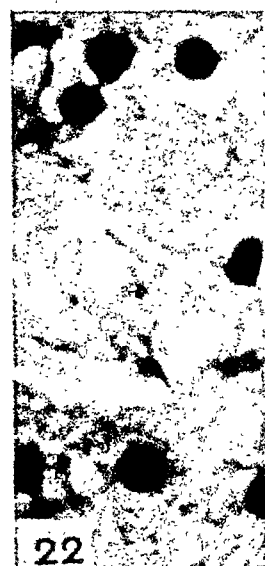
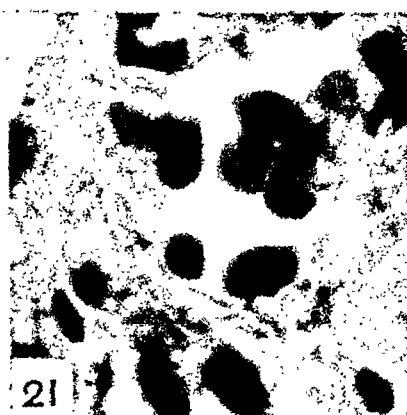
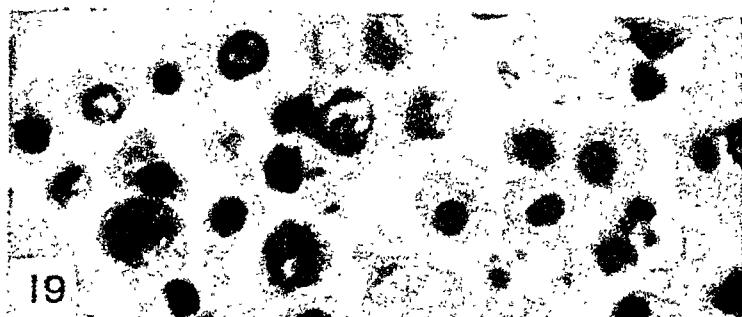
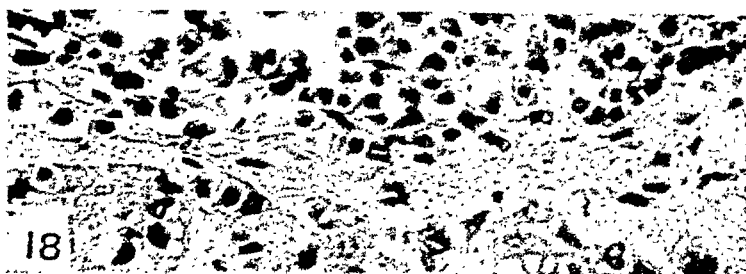
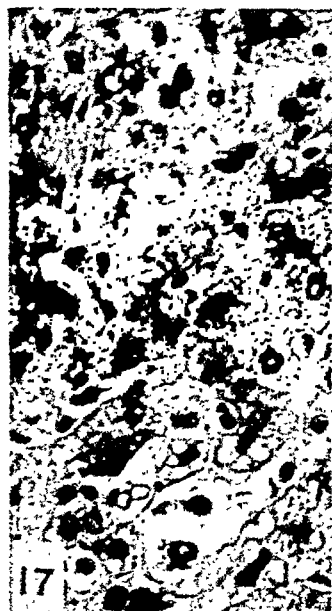
Fig. 21. Small lymphocytes in proliferating connective tissue from the cirrhotic area represented in Fig. 20. $\times 1200$.

Fig. 22. Necrotic hepatic cells from a hamster having a tumor for sixty-six days. $\times 1200$.

Fig. 23. Pyknosis and chromatolysis of lymphocytes and polymorphonuclear leukocytes in the periportal connective tissue of a hamster having a tumor for fifteen days. Feulgen stain. $\times 1200$.

Fig. 24. Hepatic cells of a hamster subjected to inanition for seventeen days. Note the large cytoplasmic vacuoles. $\times 1200$.

Fig. 25. Hepatic cells of a hamster having a tumor for fifty-one days showing the more usual condition of absence of vacuoles in the cytoplasm of the hepatic cells. $\times 1200$.



tion and as late as 158 days after implantation in one animal that had been subjected to two subtotal removals of the tumor; however, hemopoiesis did not occur in all animals having subtotal removal of the tumor, and it was completely absent in the livers of 23 of the 58 animals having the implant for more than eighteen days. The occurrence of hemopoietic foci was not related to the age of the animal at the time of implantation or to the presence of cirrhosis or necrosis of the liver (Table 2).

The cells that were present in hemopoietic foci in the liver (Fig. 26) were similar to those present in the bone marrow (Figs. 27, 28). In the liver, mitoses were numerous in cells that were identified as myelocytes (Figs. 32, 33). Small lymphocytes and plasma cells were scattered throughout the foci, as in the bone marrow of hamsters, and megakaryocytes were also present.

Both myelopoiesis and erythropoiesis occurred in the periportal areas. Myelopoiesis was decidedly the more frequent, cells of the erythropoietic series being scattered singly throughout the foci rather than being in aggregates as were the developing cells of the leukocytic series. Development of the polymorphonuclear leukocytes could be traced by variations in the nucleus from nuclei having a small central vacuole similar to that of a proerythroblast of Maximow and Bloom²⁵ to the typical polymorphonuclear form. The intermediate stages of the ring, or "doughnut", nuclei as described by Simonds³⁰ and Parsons²⁹ for the mouse, were very numerous in myeloid foci in the liver of the hamster (Figs. 30, 31).

Lymphoid nodules in the liver. Lymphocytes were very conspicuous in sections of the liver stained by the Feulgen method because of the greater content of thymonucleic acid in their nuclei than in the nuclei of hepatic cells (Fig. 29), and the lymphoid tissue was, therefore, clearly defined and readily detected (Figs. 6, 7). Lymphoid nodules were present in 14 of the 70 hamsters having tumors (Table 2), but no nodules were found in any of the 9 hamsters having implants less than five days. These nodules were not present in the livers of any of the 20 control hamsters, 8 hamsters subjected to prolonged inanition, or in the livers of the normal guinea pigs, the muskrat, or the rats examined. These

PLATE 4

Tissues in all figures, except 29, 31, 32, 33, were stained with iron-hematoxylin and eosin. All figures are $\times 1200$.

Fig. 26. Two megakaryocytes in the hemopoietic tissue occurring in the periportal connective tissue of a hamster having a tumor for twenty-two days.

Figs. 27 and 28. Two sections of bone marrow from a hamster having a tumor for fifty-six days.

Fig. 29. Lymphocytes in blood sinusoids of the liver of a hamster having a tumor for thirteen days. Feulgen stain.

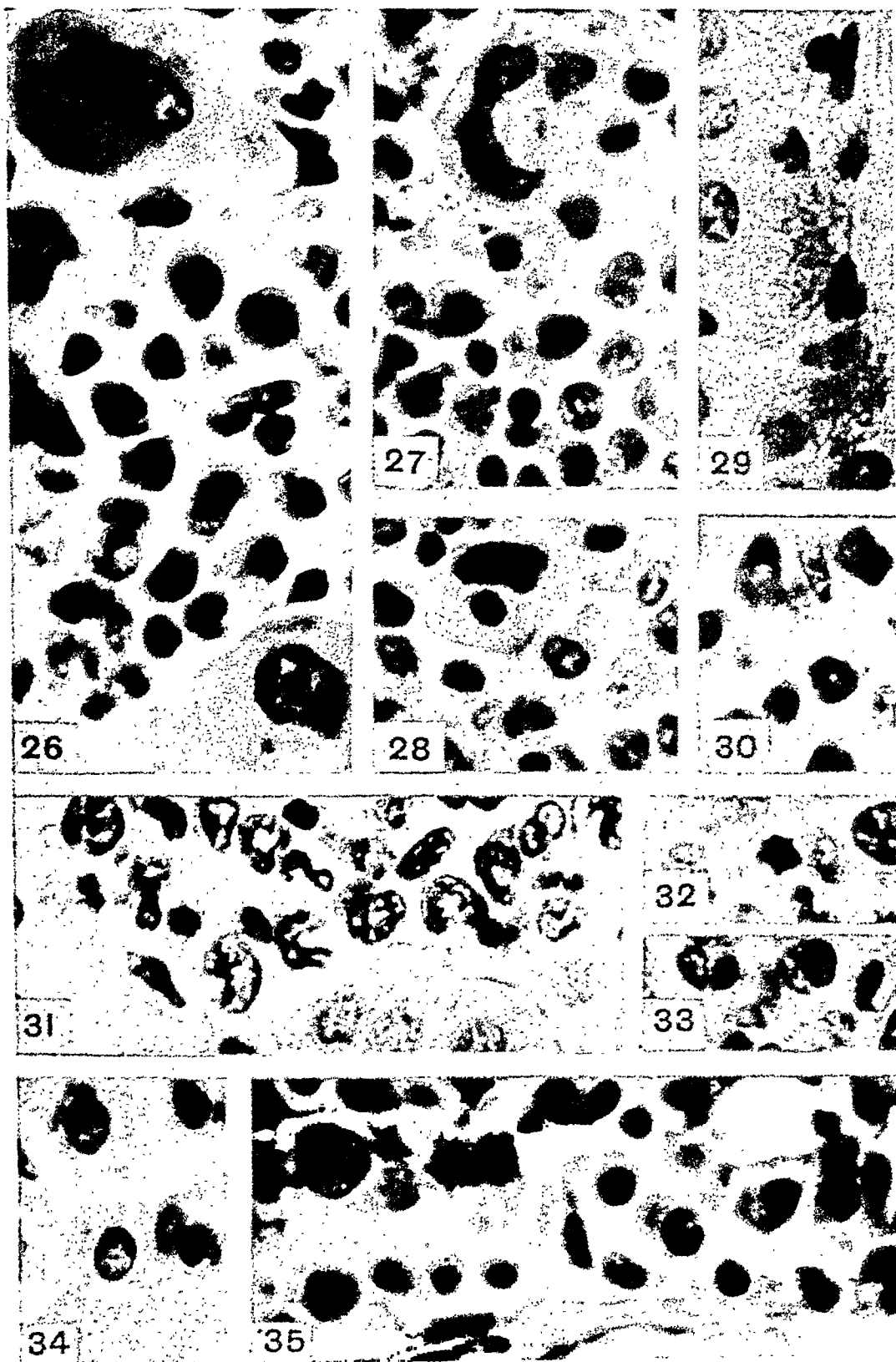
Fig. 30. Hemopoietic tissue from the liver of a hamster having a tumor for twenty-two days. Note the young (doughnut) nuclei and the two plasma cells.

Fig. 31. Several stages in the formation of nuclei of polymorphonuclear leukocytes. From the liver of a hamster having a tumor for twenty-two days. Feulgen stain.

Figs. 32 and 33. Mitoses from a hemopoietic area in the liver of the same hamster as 31. Feulgen stain.

Fig. 34. A plasma cell embedded within a hepatic cell and one surrounded by a large clear space in the liver of a hamster having a tumor for fifty-one days.

Fig. 35. Sinusoids in the periportal connective tissue showing lymphocytes and plasma cells in the liver of a hamster having a tumor for fifty-one days.



observations agree with Gerlach's¹³ statement that blood-forming cells do not occur in the livers of normal guinea pigs and with Maxia's²⁴ generalization that lymphoid infiltrations are seldom found in the livers of normal mammals.

The presence of lymphoid nodules in the hepatic parenchymas in the 14 tumor-bearing hamsters did not appear to be related to degenerative changes in the liver or to the extension of hemopoietic foci. The liver of only 1 of these 14 animals contained necrotic areas, but hemopoietic foci were present in the periportal areas of 12 of the 14 livers that contained lymphoid nodules. That the lymphoid nodules are not precursors of hemopoietic foci is indicated by the fact that hemopoiesis was limited to the extended periportal areas; while the lymphoid nodules occurred within the hepatic parenchyma (Figs. 6, 7) as described by Dawson⁷ in the liver of *Necturus*.

The lymphoid nodules in the liver are similar in structure to those in the axillary and mesenteric lymph nodes in that practically all the cells within the structure are small and large lymphocytes, while polymorphonuclear leukocytes are very rare and plasma cells are found chiefly around the periphery of the nodules. Since numerous mitoses of large lymphocytes occur within the nodule, it seems that the increase in size of the nodules is by local proliferation following apparent proteolytic action of the plasma cells upon the surrounding hepatic cells. This apparent proteolytic action makes possible an increase in space into which the nodules may extend.

DISCUSSION

The presence of periportal infiltration in the liver of each of the 70 tumor-bearing hamsters may be explained by lymphocytes and plasma cells having a part in protein metabolism. In this case the initial infiltration would perhaps be due to catabolism of the necrotic tissue that was injected, and the subsequent progressive infiltration is due to the more rapid catabolic processes caused by the rapidly-growing and necrotic tumor tissue (Stengel and Fox³¹). Drinker and Yoffey⁹ have reviewed the work of several investigators who maintain that lymphocytes contain proteolytic enzymes. The observations made in this work on the proteolytic activity of plasma cells give evidence indicating that plasma cells also produce similar enzymes.

There are several other possible explanations for this infiltration. A chronic inflammatory reaction, with infiltration of lymphocytes and plasma cells, could also be produced in the liver from toxic products of the necrotic tissue. Obstruction of lymphatics leading from the liver would cause aggregation of lymphocytes and plasma cells in the liver. The course of lymphatics in the liver has not been satisfactorily established, but no evidence from this work indicates that there were obstructions. Another problem in this assumption lies in the fact that neither the source of plasma cells nor their course in the liver is known.

SUMMARY

Lymphocytes and plasma cells infiltrated the periportal and periductal connective tissue in the livers in all of the 70 hamsters that had been subpannicularly implanted in the lumbar region with a mixed-cell sarcoma.

The initial periportal infiltration, which varied according to the amount and condition of the tumor tissue implanted, began in the host within twenty-four hours after implantation.

Extramedullary hemopoiesis in the periportal connective tissue of the liver occurred as early as eighteen days after implantation in one hamster and was present in 34 of the 70 tumor-bearing hamsters.

Lymphoid nodules were present in the parenchyma of the livers in 14; cirrhosis, in 3; and necrosis, in 3 of the 70 hosts.

Periportal infiltration, hemopoietic foci and lymphoid nodules did not occur in any of the 20 control hamsters or in the livers of the 8 hamsters subjected to inanition.

Plasma cells in the periportal connective tissues and those surrounding lymphoid nodules invaded contiguous hepatic cells and destroyed them apparently by secreting proteolytic enzymes.

Acknowledgment. The photographs were made by Mr. Glenn E. Mills, University of Colorado School of Medicine.

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EFFECT OF INTRAVENOUS ADMINISTRATION OF DEXTRAN, A MACROMOLECULAR CARBOHYDRATE, IN ANIMALS¹

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Dextran is a macromolecular carbohydrate which is formed by the polymerizing action of the bacterium *Leuconostoc mesenteroides*, on sucrose.^{6, 7, 8} Swedish investigators⁴ recently reported favorable results on a large amount of clinical material with the use of Dextran solution as a plasma substitute. This suggested the need for a thorough experimental study on the effect of the intravenous injection of macromolecular substances in general. Such a study would supplement investigations made on gum acacia, pectin and gelatin and especially the extensive studies of Hueper^{11, 12, 13} on macromolecular substances in general. Dextran deserves special interest since it represents an easily split carbohydrate polymer chemically related to glycogen or starch. The investigations undertaken were similar to previous ones on gelatin and pectin.¹⁵ They included the ability of Dextran solution to produce hemodilution, the effect on the blood, on renal and hepatic functions, the histologic changes produced by repeated administration and, finally, investigations as to possible antigenicity of Dextran. The latter appeared desirable in view of studies showing its relationship to the carbohydrates in pneumococci.

MATERIAL

The material used (Macrose) was a partly hydrolyzed Dextran. (It was provided in 6 per cent solution in distilled water by Schenley Laboratories in 500 cc. flasks filled and sterilized by Baxter Laboratories.) Its average macromolecular size was considered more suitable for use in fluid-replacement therapy than the original Dextran. It represented a water-clear fluid of syrupy consistency, perfectly liquid even at ice box temperature. The experiments were carried out on a total of 8 dogs, 26 rabbits and 14 guinea pigs.

RESULTS

Hemodilution experiments. Rabbits and dogs were injected with 15 cc. of the Dextran solution per Kg. body weight and the changes in hemoglobin, red blood cell count, hematocrit, total protein and white blood cell count were determined thirty minutes, three hours and twenty-four hours after the administration. As seen in Table 1, a significant reduction occurred in each of the factors listed. These changes were somewhat less marked three hours after

¹ Supported by a grant from Schenley Laboratories, Inc., New York, N. Y. Received for publication, September 2, 1947.

the administration, and were only one-half as marked twenty-four hours later. The changes in the values of the different factors were parallel except for the white blood cell count which returned more quickly to normal, showing that the changes were not due merely to the hemodilution. When the administration was repeated at one day intervals, a cumulative effect of the administration upon the hemodilution was noted, as seen in Fig. 1.

Sedimentation rate. The sedimentation rate rose in all instances following the administration of Dextran. At no time was rouleau formation demonstrated in spite of the elevated sedimentation rates. Repeated injections at short in-

TABLE 1

HEMODILUTION EXPERIMENTS IN DOGS AND RABBITS. 15 CC. OF DEXTRAN PER KILOGRAM OF BODY WEIGHT WAS ADMINISTERED INTRAVENOUSLY

DOGS							RABBITS					
Determination	Number of experiment	Number of animals tested	Average level before determination	Average percentage in change at indicated time after administration			Number of experiment	Number of animals tested	Average level before determination	Average percentage in change at indicated time after administration		
				1/2 Hour	3 Hours	24 Hours				1/2 Hour	3 Hours	24 Hours
Hemoglobin	6	2	12.5%	-21.1	-13.9	-13.0	11	4	10.4%	-18.4	-16.8	-13.2
Red blood cells	6	2	5.31 (millions)	-21.1	-11.2	-7.4	11	4	4.71 (millions)	-19.2	-16.2	-9.6
Hematocrit	14	5	38.4%	-19.8	-15.3	-10.5	11	4	37.9%	-23.7	-23.3	-15.3
Total protein	18	5	5.51%	-17.8	-16.8	-3.8	9	4	4.6%	-27.2	-19.2	-13.0
White blood cells	7	3	16,193	-24.3	+4.5	+16.5	11	4	10,909	-42.4	-18.0	+0.8
Sedimentation rate	6	2	0.7 mm./hr.	+14.0	+29.3	+20.8	11	4	9 mm./hr.	+19	+32	+10
Coagulation time	3	1	80 seconds	+30.	+15	0.						
CO ₂ combining power	13	3	49%	+0.8	0	-1.2						

tervals also revealed a cumulative effect upon the sedimentation rate with gradual return to lower levels (Fig. 1).

Influence of repeated administrations. A series of animals received repeated injections of 15 cc. of Dextran per Kg. body weight three times a week for several weeks, up to a total of 46 injections. Despite the very large amounts of Dextran used, no significant changes were produced as far as blood count, hematocrit, hemoglobin and total protein concentration were concerned. At regular intervals during the period when the injections were being administered, the following determinations were made to test for hematologic changes or aberrations in renal or hepatic function: red and white blood cell counts, hemoglobin, hematocrit, bleeding time, clotting time, sedimentation rate, total protein, blood sugar, albumin-globulin ratio, cholesterol, cholesterol esters, thymol turbidity, serum

alkaline phosphatase, urea clearance, nonprotein nitrogen, urinary albumin, urinary sugar, urobilinogen, stool urobilinogen, serum bilirubin and prothrombin time. Table 2 summarizes some of the results obtained showing that in some of the animals a slight anemia resulted whereas all the other tests revealed no significant alteration.

Dextran blood and urine levels. To compare the degree of hemodilution with the blood Dextran level and to study its speed of disappearance from the blood

TABLE 2

INFLUENCE OF REPEATED ADMINISTRATION OF DEXTRAN OVER PROLONGED PERIOD UPON VARIOUS BLOOD FACTORS IN DOGS AND RABBITS

DOGS							RABBITS							
Animal number		4	1	5	3	2	7	1	12	11	13	14	3	4
Weight in Kg.		24.4	13.6	4.54	5.06	5.2	10.0	4.25	2.04	2.04	4.8	4.8	4.55	3.32
Duration of experiment in days		129	20	77	62	60	34	112	68	46	37	57	69	36
Total amount injected in cc.		16,839	6750	2181	2925	1704	1350	2724	765.6	520.2	1150	1150	1572	948
Number of injections		45	34	32	25	23	9	41	25	17	25	25	29	16
RBC	Control	3.59	7.05	9.9	4.05	4.45	5.91	5.37	4.65	6.04	5.42	4.81	5.35	5.12
	Final	4.54	6.62	3.94	6.44	5.39	5.52	3.48	5.41	5.92	4.45	3.91	3.76	3.44
Hb., gm. per 100 ml.	Control	7.5	16.9	15.2	10.3	10.6	13.7	12.5	13.0	19.5	15.0	13.2	11.4	19.8
	Final	8.5	14.	11.5	14.8	12.5	12.7	10.3	11.6	19.5	11.0	10.0	5.5	6.6
Hematocrit	Control	29.	53.	49.	33.	38.	43.	42.2	39.	38.0	44.	42	39.	35.
	Final	31.2	48.	40.	45.	47.	41.7	37.	41.	33.1	38.	32.	25.	21.5
Sedimentation rate	Control	1.	0.	1.	1.	1.5	1.	0.	2.	1.	5	1.	0.	0.
	Final	2.9	3.	25.	1.	0.	22.	1.	1.	2.	6.	2.	25.	71.
Total protein in gm.	Control		4.89	4.9		6.4	4.77	4.4	5.3	5.8	7.09	7.11	6.25	
	Final	6.39	4.8	6.34	6.59	5.91	4.5	5.4	5.45	5.31	7.45	5.41	4.66	3.24
Alkaline phosphatase	Control	3.4	7.8	7.1	1.8	2.4	7.7	2.1	9.1	4.5	5.9	7.4		
	Final	5.1	6.7	10.8	3.5	3.0	12.8	1.2	3.2	3.2	9.7	2.2	4.5	
Thymol turbidity	Control	0.6	7.3	0.6	0.7	5.0	3.0	0.8	0.6	0.3	2.4	1.5		
	Final	3.	3.2	0.6	7.9	5.0	3.5	0.8	1.0	0.6	1.0	1.3	1.6	
Nonprotein nitrogen, mg. per 100 ml.	Control	31.	22.	33.	25.	40.	37.	35.	33.5	33.5	43.7	31.	40.	38.
	Final	37.5	45.	34.8	52.	39.	33.	35.	28.0	32.5	37.2	49.	29.	31.

as well as its excretion in the urine, a method for Dextran determination was devised which rendered reproducible results in both blood and urine. The previously described method of Hint,¹⁰ although reliable for blood, could not be successfully applied to the urine. The method used by us is based upon precipitation of macromolecular carbohydrates such as Dextran, by alkaline copper sulfate, as used in the method of Hint, with redissolving of the precipitate in distilled water. In the resulting solution, the carbohydrates are determined by the indol-sulfuric acid method of Dische and Popper.² Of Dextran added to

serum and urine, about 90 per cent was recovered. In the urine, under basic conditions, a small amount of macromolecular carbohydrate of dextran character was found which would react as Dextran with the method used.

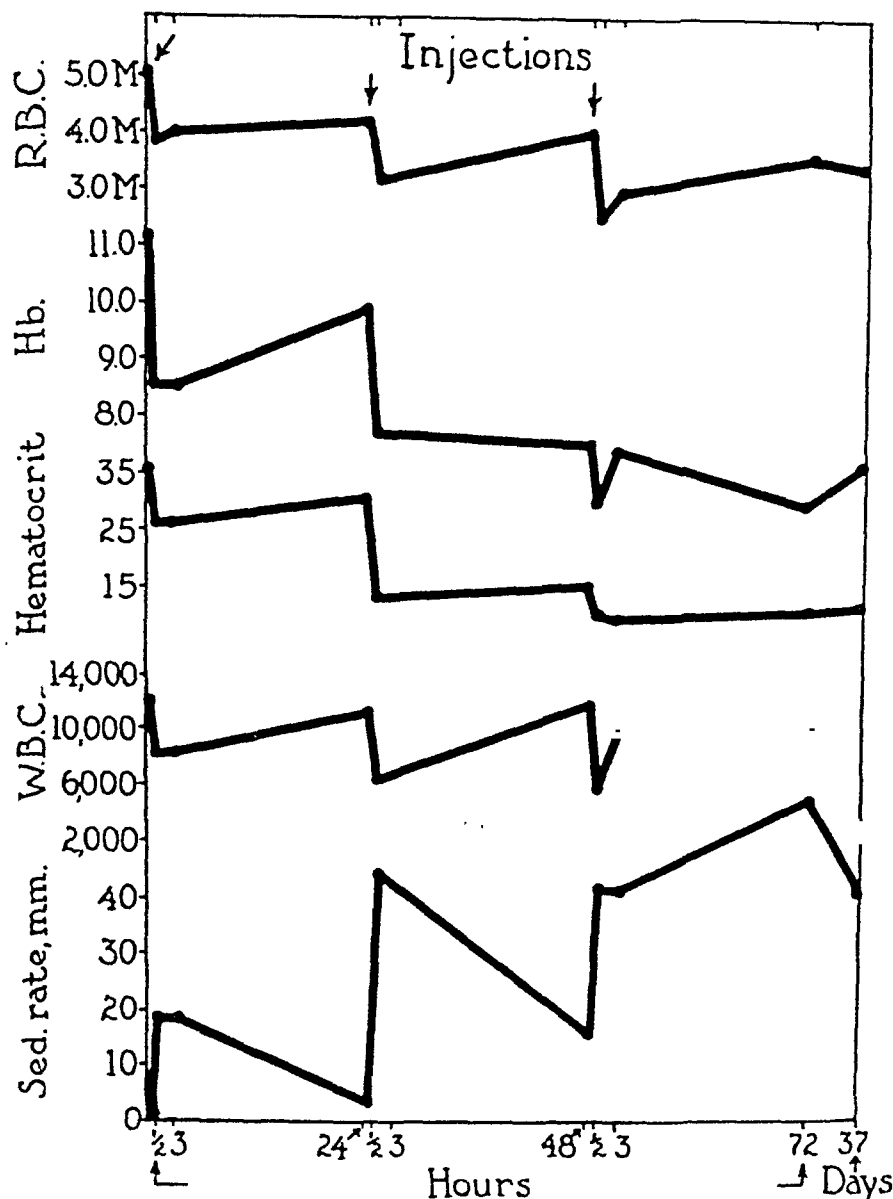


Fig. 1. Changes of red cell count, hemoglobin, hematocrit, white blood cell count and sedimentation rate in a dog after repeated injections of 15 cc. of Dextran 80 per cent solution per Kg. body weight.

Blood and urine levels of Dextran were determined in 2 dogs following the administration of 15 cc. of Dextran per Kg. body weight. After several days, when the blood level had returned to zero, two additional injections were given

at one day intervals which were followed by determinations until return to zero. The experiments gave equal results, one of which is illustrated in Fig. 2. As expected, the serum Dextran concentration is high five minutes after the intravenous injection. A rapid drop follows after the first hour after which time the drop becomes less rapid, but twenty-four hours later, the serum level still shows about one-fourth of the original elevation. After repeated injections, the serum level is maintained much longer and returns to zero only after five days. The bulk of the urinary excretion does not occur within the first three hours, but within the first or second day. The excretion is maintained for several days. The total amount excreted is only a small fraction, about 6 per cent of the total amount injected. Copper reducing carbohydrates were at no time excreted.

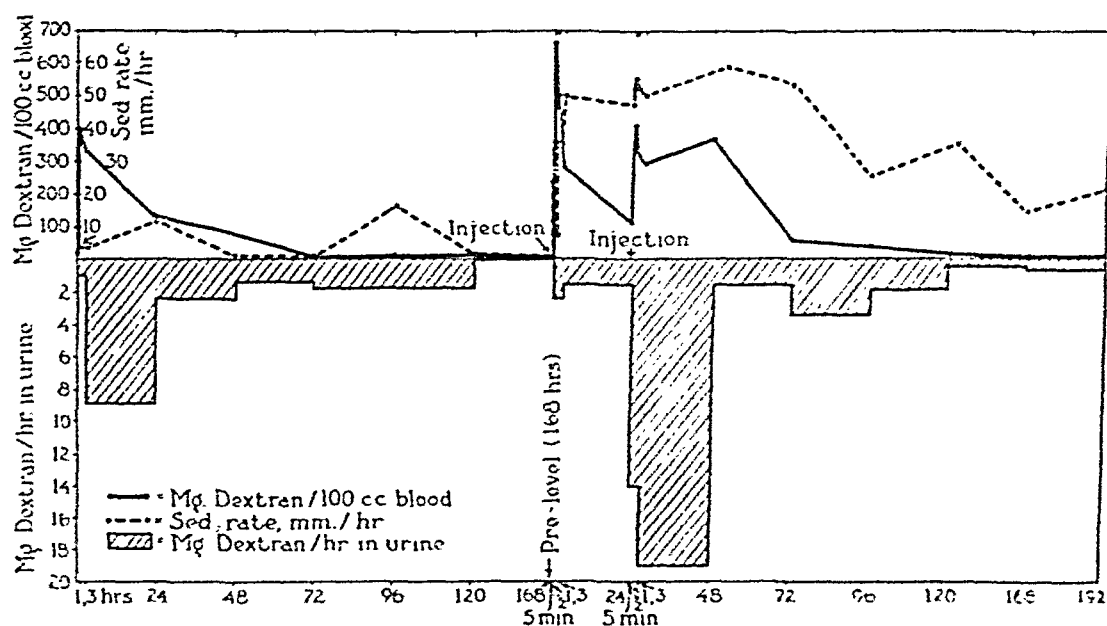


FIG. 2. Blood Dextran level, urinary Dextran excretion and sedimentation rate following repeated injections in a dog of 15 cc. of Dextran solution per kilogram body weight.

Antigenicity experiments. Anaphylaxis experiments on 14 guinea pigs previously sensitized by intraperitoneal injection of Dextran solution, revealed negative results after intracardiac injection. Also, precipitin and complement-fixation tests of the serum of animals listed in Table 2 with Dextran were negative, as were conjunctival tests on some of these animals.

Histologic examination. In all rabbits and dogs sacrificed less than two days after an injection of Dextran, swelling and granularity of the epithelial cells of the tubules of the kidney were seen; these were associated with the presence of granular debris or homogeneous pink-staining globules in the lumens of the tubules (Fig. 3a). In some instances marked vacuolization of the epithelium and dilatation of the tubules were noted (Fig. 3a). The changes involved in order of frequency, the proximal convoluted tubules, the distal convoluted tubules

and the loop of Henle. Special stains were negative for fat, and for glycogen or other carbohydrates (Bauer-Feulgen reaction). In the involved tubules a decrease or disappearance of alkaline phosphatase was noted. The number of injections was not necessarily of significant influence on the development of these changes and the tubules appeared normal more than five days after the last injection, even when a large number of injections had been given.

Glomerular and interstitial changes seemed to be more closely related to the number of injections. With the hematoxylin-eosin stain, the glomerular changes were characterized by homogeneous pink-staining swelling of the loops. These changes varied in severity, up to almost complete replacement or obliteration of the glomerular elements by the homogeneous areas (Fig. 3b). The glomerular and capsular epithelial cells revealed reactive hyperplasia. The homogeneous material did not give the Bauer-Feulgen reaction for carbohydrates, and glycogen and amyloid stains were also negative. With McGregor's modification of Mallory's aniline blue stain these homogeneous areas stained deep blue as for collagen or hyaline (Fig. 3c).

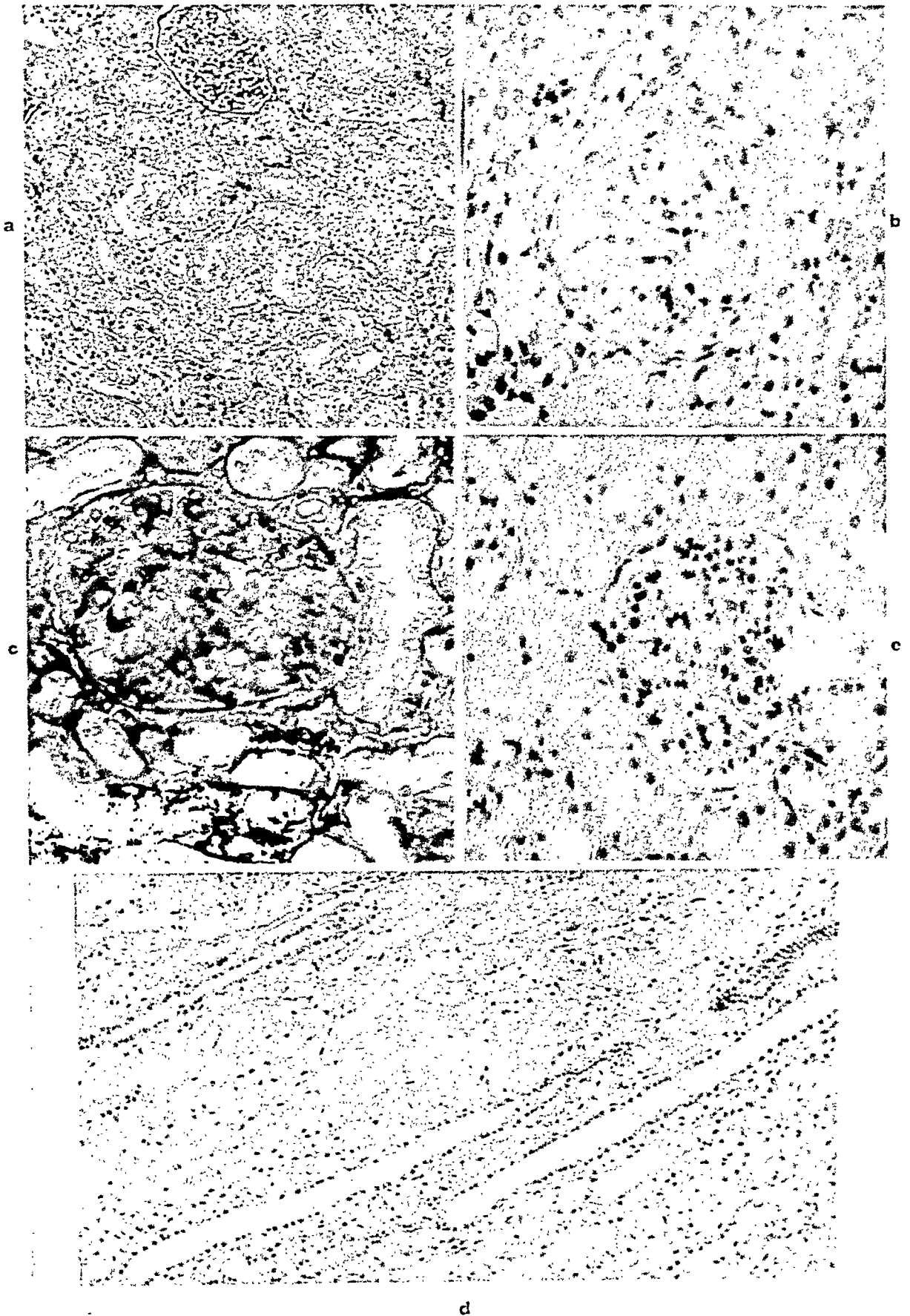
In Rabbit 4, the kidneys were grossly enlarged. Homogeneous, pink-staining material was deposited in the interstitial tissue commencing near the corticomedullary boundary, increasing toward the pyramids and decreasing around the papillae (Fig. 3d). In less involved foci it was most marked around thick walled medullary capillaries (Fig. 3d). The glomerular changes, which generally were more marked in dogs than in rabbits, decreased only slightly if some time elapsed between the last injection and the time of sacrifice of the animal.

To investigate whether preceding renal changes increase the deposition of the pink-staining material, 6 mg. of Salyrgan per Kg. body weight was injected intravenously into each of 8 rabbits. Four of them were given 15 cc. of Dextran per Kg. on the third day after the Salyrgan administration and on alternate days thereafter for a total of four injections. Two days later the animals were sacrificed and all revealed various degrees of a mercury induced toxic nephrosis. In the Dextran treated animals thickening of the glomerular loops was noted (Fig. 3e) which, however, was comparable in degree to that found in rabbits receiving similar amounts of Dextran without Salyrgan administration.

The remaining organs of the examined animals failed to reveal characteristic histologic changes. Histiocytic reaction or arterial intimal proliferation, as described after intravenous injection of other macromolecular substances,¹⁴ was absent.

FIG. 3. Photomicrographs of kidneys of animals which had received Dextran injections. *a.* Dog No. 3, granularity of cytoplasm of convoluted tubules, with amorphous and reticular debris in lumens, some of which are dilated. Hemalum and eosin. $\times 80$. *b.* Rabbit No. 4, almost complete hyalinization of a glomerulus; hydropic vacuolization of convoluted tubules. Hemalum and eosin. $\times 230$. *c.* Rabbit No. 4, thickening of glomerular loops. Mallory connective tissue stain (McGregor modification). $\times 230$. *d.* Rabbit No. 4, homogeneous interstitial hyalin transformation in medullary portion of kidney; tubular atrophy and thickening of the capillary wall. Hemalum and eosin. $\times 80$. *e.* Rabbit treated with Dextran and Salyrgan, moderate thickening of basement membranes of glomerular loops. Hemalum and eosin. $\times 230$.

FIG. 3



General constitutional manifestations. No alterations were noted in any of the treated animals, even in those receiving the reported high amounts of Dextran. They gained weight and no anorexia was noticed.

COMMENT

Almost nothing in the studies presented on rabbits and dogs would contraindicate the use of Dextran as a plasma substitute provided it would meet clinical standards in human beings; only the significance of the glomerular changes deserves further consideration before employing the substance. It is an efficient hemodiluting agent which rather rapidly disappears from the blood stream although only a small part is excreted by the kidneys. Little cumulative effect is noted if repeated injections are given and no untoward effects on renal or liver function were noted. The rise in the sedimentation rate is a characteristic sequela of the injection of any macromolecular substance.⁵ Rouleau formation which may lead to dangerous thrombus formation,¹⁵ was never encountered. The mild anemia occasionally seen after prolonged administration is possibly still the result of protracted hemodilution.

An explanation, however, may be required for the marked renal tubular changes noticed which are apparently transient in character. The disappearance of alkaline phosphatase from the renal tubules has been considered as evidence of functional impairment of the kidney;⁹ however, the laboratory studies on the animals *in vivo* revealed no significant alterations, and in this instance, therefore, the histologic investigation appears more sensitive than the functional studies. The lesion appears to be identical to the transient changes following administration of gelatin,¹⁸ which have been called gelatin nephrosis.² They are also identical with the changes observed in the human kidney following the administration of sucrose.¹ In the vacuoles of the tubular epithelium Dextran could not be demonstrated by specific carbohydrate stains; however, it may have been dissolved during the embedding process. This Dextran nephrosis appears to be another example of the transient renal tubular changes following the intravenous administration of larger, nontoxic molecules which are filtered through the glomeruli and, on reabsorption through the tubules, cause hydration of their epithelium.

The deposition of the pink-staining material in the glomeruli and in the one instance in the interstitial tissue, is obviously of greater significance since it does not appreciably diminish after discontinuance of the Dextran administration. In view of the negative histochemical reactions this material is obviously not Dextran itself but possibly a protein precipitate resulting from temporary deposition of a large, water-binding carbohydrate molecule. The blue color in the Mallory reaction supports this hypothesis. The lesion may also be explained by deposition of large molecules within the wall of the glomerular tuft leading to a secondary protein precipitation. The lesion in the Dextran animals did not impair renal function as indicated by nonprotein nitrogen or urea clearance and was not enhanced by preceding mercurial nephrosis.

The fate of Dextran in the body is not quite clear. Very little, about 6 per

cent, was excreted in the urine as macromolecular carbohydrate and nothing as reducing sugar; no evidence for significant deposition in any organ was found. One, therefore, can assume that the macromolecular substance is broken down to small carbohydrates more easily assimilable in the body.

From a general standpoint it is noteworthy that injections of relatively large amounts of a macromolecular substance repeated at short intervals for a long period do not interfere with the general condition and well-being of an animal, even when more than 1 Kg. of the solid material is given to a dog weighing 24.4 Kg. These large amounts do not produce any clinical or anatomic changes of the liver, although this organ probably carries a great load in disposing of this material. This macromolecular carbohydrate which apparently is easily split, may, therefore, be added to the series of substances tested by Hueper and others as possible plasma substitutes. This material seems to represent the most innocuous substance of the series.

SUMMARY

On intravenous administration, a 6 per cent Dextran solution is an effective hemodiluting agent in dogs and rabbits. The blood Dextran level decreases rapidly within a few hours and after twenty-four hours is about one-fourth the original elevation.

Only a small fraction of Dextran administered is excreted in the urine as macromolecular or other carbohydrate. This suggests that the greatest part is broken down and assimilated by the body.

Histologically, transient changes in the renal tubules were found similar to the ones which follow the administration of gelatin or sucrose. Although the alkaline phosphatase stain showed morphologic impairment of the renal tubules, no evidence was found of impaired function. After administration of larger amounts of Dextran, a peculiar material was deposited within the glomerular loops but this deposition did not disturb renal function.

Except for an occasional slight anemia, no significant permanent changes were encountered in the blood, even after frequently repeated intravenous administration of Dextran. The intravenous administration of exceedingly high amounts of this easily assimilable macromolecular substance (e.g., more than 4 per cent of the body weight in solid material in 126 days) did not interfere with the well-being of the experimental animal.

Judged from these animal experiments, Dextran may represent a good plasma substitute and should be subjected to clinical tests in human patients.

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VERIFICATION ANTIGEN FOR THE IDENTIFICATION OF PSEUDOSYPHILITIC REACTIONS OF SERUM*

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The specificity of tests for syphilis has never been regarded as absolute and this has been shown to be especially true during the past few years. Since the early days of serodiagnosis of syphilis, it has been known that conditions other than syphilis, such as yaws, leprosy, scarlatina, malaria, typhus, pinta, pregnancy and malignant tumors produce in some patients serologic tests which are positive in varying degree. In recent years, the following conditions have been added to the list: hyperproteinemia,³ smallpox vaccination,^{1, 4, 10, 13} infectious mononucleosis,^{18, 22} meningitis¹⁹ and repeated blood donation.^{5, 6, 7} These observations have been discussed and partly confirmed by many authors.^{2, 7, 9, 12, 14-17, 20-22} I would suggest that such a reaction be spoken of as a "pseudosyphilitic", rather than as a false positive, reaction. The term "pseudosyphilitic" seems to be better since it signifies that the test imitates a positive test for syphilis but is not caused by syphilis.

Verification tests to confirm the correctness of a dubious positive reaction and to exclude a possible nonspecific reaction were first tried by Wassermann (1921), later by Witebsky (1931) and recently by Kahn (1940). The verification antigen described in this paper gives positive results with some nonsyphilitic serums, as in malaria, and negative results with syphilitic serums.

METHOD

In work carried on in the preparation of a cancer antigen, various solvents were used in the preparation of extracts of neoplastic and "pregnancy" tissues. "Pregnancy" tissue included pregnant uterus, fetus, decidua, cord and placenta. It must be stated that in order to exclude the interference of syphilis-positive serums, the tissue used for the preparation of a cancer antigen is first extracted completely with lipoid solvents such as alcohol, acetone and petrol ether. The aqueous extraction of the remaining tissue gives an antigen which does not react even with strongly positive syphilitic serums.

A selected group of about 200 serums from approximately 20,000 serums routinely tested in a period of twelve months was used for the following analysis. These selected serums were all positive with the Kline antigen and negative with the Pangborn antigen. In tracing the clinical diagnoses in the patients from whom these serums were taken, it was found that none of the patients had a history of syphilis or any evidence of the disease except for the positive serologic test. These 20,000 routine serums were obtained from all departments of the hospital including the Out-patient Department. This means that most of them were not syphilitic. When differing results were obtained with the Kline and

* Received for publication, July 10, 1947.

Pangborn antigens, the serum was tested with our verification antigens. The results obtained with our aqueous antigens were far superior to those obtained with our alcoholic antigens and the exclusive use of these aqueous antigens can be recommended in such dubious cases.

The verification antigen can be made as follows:

1. Dried, powdered tissue (pregnancy tissue, comprising pregnant uterus, fetus, decidua, cord, placenta, giving better results than beef heart powder)

TABLE 1

COMPARISON OF READINGS OF SEROLOGIC SLIDE TESTS USING KLINE AND PANGBORN ANTIGENS WITH SLIDE TESTS USING VERIFICATION ANTIGENS PREPARED FROM CANCEROUS TISSUES

ANTIGEN USED*		CLINICAL DIAGNOSIS						
		Subacute Endocar- ditis	Uterine Blood from Normal Control	Uterine Blood from Normal Control	Carcin- oma of Sig- moid	Syphilis	Pregnan- cy	Pregnan- cy
Kline antigen	Diagnostic	++++	-	-	-	+	-	-
	Exclusion	++++	-	-	-	+++	-	-
	Control	++++	-	-	-	++++	-	-
Pangborn antigen	Diagnostic	-	-	-	-	+++	-	-
	Exclusion	-	-	-	-	++++	-	-
Carcinoma of colon no. 71/1		-	±	++	-	+	++++	+++
Carcinoma of colon no. 71/3		-	+++	++	-	±	-	+++
Carcinoma of colon no. 72 1/8		±	-	+++	-	+	-	++++
Carcinoma of colon no. 72 3/8		++++	++		+++	-	++++	+
Carcinoma of colon no. 91 1/8		-	+++	+++	-	+	++	-
Carcinoma of colon no. 91 3/37		-	+++	++++	-	++++		++++
Carcinoma of breast 1/8		-	-	+++	-	-		-

± equals doubtful.

* Carcinoma of colon no. 71/1 was an acetone-soluble, petrol-ether-insoluble, alcohol-soluble antigen from tissue of carcinoma of colon no. 71; carcinoma of colon no. 71/3 was an acetone- and petrol-ether-insoluble, alcohol-soluble antigen from carcinoma of colon no. 71; carcinoma of colon no. 72 1/8 was an acetone-soluble, petrol-ether-insoluble, alcohol-soluble antigen from carcinoma of colon no. 72 prepared at 8 C.; carcinoma of colon no. 72 3/8 was an acetone and petrol-ether-insoluble, alcohol-soluble antigen prepared from carcinoma of colon no. 72 at 8 C.; carcinoma of colon no. 91 1/8 was an acetone-soluble, petrol-ether-insoluble, alcohol-soluble antigen prepared from carcinoma of colon no. 91 at 8 C.; carcinoma of colon no. 91 3/37 was an acetone- and petrol-ether-insoluble, alcohol-soluble antigen prepared from carcinoma of colon no. 91 at 37 C.; carcinoma of breast 1/8 was an acetone-soluble, petrol-ether-insoluble alcohol-soluble antigen prepared from carcinoma of the breast at 8 C.

is completely extracted with alcohol, next with petrol ether and then with acetone. The remaining tissue must be dried and extracted with distilled water plus 0.3 per cent phenol. For each 10 gm. of dried tissue 200 cc. distilled water with 0.3 per cent phenol is added and extracted in an incubator at 37 C. for twenty-four hours. It is then filtered and kept for at least two weeks in the refrigerator before evaluation.

2. Dried, powdered tissue is first extracted with petrol ether, next with acetone

TABLE 2

COMPARISON OF READINGS OF SLIDE TESTS USING KLINE AND PANGBORN
ANTIGENS WITH SLIDE TESTS USING VERIFICATION ANTIGENS
PREPARED FROM HUMAN AND BEEF BILE

ANTIGEN USED*		CLINICAL DIAGNOSIS				
		Epidermoid Carcinoma of Cervix; Fibroma Uteri	Carcinoma	Syphilis	Medical Observa- tion	Postappen- dectomy State
Kline antigen	Diagnostic	+++	--	+++++	--	--
	Exclusion	+++	--	+++++	--	--
	Control	+++	--	+++++	--	--
Pangborn antigen	Diagnostic	±	--	+++++	--	--
	Exclusion	--	--	+++++	--	--
HB Iw		+++++		--	±	--
HB IIw		+++++		--	+++++	+++++
HB IIIw		+		--	+++++	+++++
HB IVw		--	--		+	--
HB Vw			+++++	--	+++++	--
BB Acw			+++++	++	+++++	--

± equals doubtful.

* HB antigens were prepared from dried human bile after extraction with petrol ether, acetone and alcohol. The remainder (alcohol-insoluble) was dissolved in distilled water plus 0.3 per cent phenol. Antigens labeled Iw, IIw, IIIw, IVw and Vw were prepared from human bile derived from various patients; BB Acw was an aqueous extract of beef bile after treatment with acetone, petrol ether and alcohol.

TABLE 3

COMPARISON OF READINGS OF SLIDE TEST USING KLINE AND PANGBORN
ANTIGENS WITH SLIDE TESTS USING VERIFICATION ANTIGENS
PREPARED FROM HUMAN BILE

ANTIGEN USED*		CLINICAL DIAGNOSIS						
		Postpar- tum State	Postpar- tum State	Postpar- tum State	Preg- nancy	Preg- nancy	Medical Observa- tion	Syphilis
Kline antigen	Diagnostic	--	--	--	--	--	--	+++++
	Exclusion	--	--	--	--	--	+++	+++++
	Control	--	--	--	--	--	+++++	+++++
Pangborn antigen	Diagnostic	--	--	--	--	--	--	+++++
	Exclusion	--	--	--	--	--	--	+++++
HB IVw		+++++	+++++	+++++	+++	+++++	+++	--
HB VIw		+++++	+++++	+++++	++	+++++	+++	--
HB VI Ale 21		--	--	++		+++++	+++++	--
HB VI Ale 50		--	--	++		+++++	+++++	--

* Antigens HB IVw and HB VIw were prepared as described under Table 2; antigen HB VI Ale 21 was an alcoholic extract of human bile prepared at room temperature (21 C.); after filtration, the remainder was placed in an incubator at 50 C. with alcohol and extracted over-night and labeled HB VI Ale 50.

and then with alcohol. From the remaining tissue, the antigen may be prepared as stated above.

Evaluation of antigen. Prepare a 1:5 dilution of the antigen with 0.9 per cent saline and dilute this further to 1:10, 1:20, 1:40 and 1:80. Use at least ten different serums for the first evaluation with each of these dilutions. The serums should include syphilitic serums of all grades of positivity, serums from pregnant women and from patients with pneumonia and tuberculosis, also normal serums. As a tentative dose, the dilution which is negative with syphilitic serums but positive with some of the other serums, especially pregnancy serums and serums from patients with cholecystitis, should be taken. After testing 40 or 50 different serums, it should be clear whether or not this special antigen can be used. A good verification antigen is one which is completely negative with

TABLE 4

COMPARISON OF READINGS OF SLIDE TESTS USING KLINE AND PANGBORN ANTIGENS WITH SLIDE TESTS USING VERIFICATION ANTIGENS PREPARED FROM BEEF HEART, PIG BILE AND HUMAN BILE

ANTIGEN USED*		CLINICAL DIAGNOSIS		
		Pregnancy	Syphilis	Syphilis
Kline antigen	Diagnostic	—	+++	++++
	Exclusion	±	++++	++++
	Control	+++	++++	++++
Pangborn antigen	Diagnostic	—	++++	++++
	Exclusion	±	++++	++++
BHW		++	±	—
PB Acw		++++	—	—
HB IVw		++++	—	—

± equals doubtful.

* BHW was a watery antigen extracted from beef heart powder following treatment with petrol ether, acetone and absolute alcohol; PB Acw was a watery antigen made of pig bile and extracted with acetone, petrol ether and alcohol; HB IVw was the same as described in Table 2.

syphilitic serums but which gives at least a 2 plus reaction with serums from patients with wasting diseases such as tuberculosis, Hodgkin's disease, carcinoma or other neoplasms, brain concussion or pregnancy.

RESULTS

In Tables 1 to 4 are listed the results of the readings of serologic tests using Kline and Pangborn antigens and verification antigens prepared from cancerous tissues (Table 1), human bile or beef bile (Table 2), human bile (Table 3), and from beef heart, pig bile or human bile (Table 4). The results are arranged so that a comparison may be made of readings with the different antigens in testing syphilitic and nonsyphilitic serums.

DISCUSSION

The introduction of the cardiolipin antigen is really a great improvement, but it is still not the final solution of the problem. Rein has pointed out in his discussion of the paper by Kline¹¹ that the cardiolipin tests have definite limitation in that false-positive and false-negative reactions may be obtained with them. He obtained frequent nonspecific (false positive) reactions with cardiolipin tests in patients with leprosy, infectious mononucleosis, yaws and pinta.

Boerner, Nemser and Stokes⁵ studied the effect of bleeding and of repeated blood donation on serologic tests for syphilis. They used two serologic tests, one a quantitative flocculation test and the other a quantitative complement-fixation test designed to determine the ability of serum to fix complement but differing from the usual methods in that the amount of complement was varied and the serum dose was kept constant. It was surprising to find that there was no significant difference in reagin (antibody) content of the serum among the 128 blood donors immediately before and immediately after venesection. Ninety-seven blood donors were tested before donation and at intervals from the fifth to the fifteenth day following donation. One showed an increase of two complement units. None showed any increase in flocculation units. The authors concluded that these experiments did not reveal the existence of a nonspecific serologic test for syphilis due to multiple blood donation or to bleeding as such. Neither can it be said that such a possibility is totally excluded.

Therefore, it seems desirable to utilize another method of control for the identification of pseudosyphilitic reactions.

SUMMARY

Verification antigens prepared as described above have been utilized to control the results obtained with the slide test using Kline and Pangborn antigens, and with the Active Method (Hecht) in the complement-fixation technic. Serums which gave strongly positive reactions with the Kline and Pangborn antigens reacted negatively to the control antigens. In cases where a difference was observed between the Kline antigen and the Pangborn antigen, the verification antigen almost always agreed with the Kline antigen, but disagreed with the Pangborn antigen. It was found that most of these serums came from pregnant women and patients having neoplasms or acute febrile conditions. When syphilis was associated with diseases such as cancer and tuberculosis, or with pregnancy, a positive reaction was obtained with Kline, Pangborn and the verification antigen. The use of these verification antigens seems to be a good control for detection of pseudosyphilitic reactions.

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CLINICOPATHOLOGIC CONFERENCE

A CASE OF REGIONAL ENTERITIS*

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CLINICAL DATA

A white male architect, 28 years old, entered this hospital on November 16, 1944, stating, "I have sprue".

Past Illness

The patient had never lived in the tropics. In 1929, at the age of 13, he entered a large hospital in Detroit because of suspected acute appendicitis. A history was obtained of recurrent attacks during the previous ten years of epigastric pain with nausea and vomiting. Some of these attacks had been preceded by intemperance in eating. Examination revealed severe tenderness in the midepigastrium and left upper quadrant, slight tenderness in the left lumbar region of the abdomen and voluntary spasm in these areas. X-ray films disclosed no evidence of organic lesion of the esophagus, stomach or duodenum, but there was irritability and sharp tenderness over the region of the duodenal bulb and some tenderness over the cecal area. The diagnosis of acute appendicitis was ruled out and he was discharged from the hospital. At the age of 17 years, he lost his mother and thereafter his diet consisted mainly of candy, cold beverages and frankfurters. He was relatively well until 1940.

Present Illness

In May 1940, the patient first developed a diarrhea which progressively increased in severity until one and one-half years later (December 1941), when he had as many as 28 bowel movements a day. The stools were frothy, soft, pale and had a very foul odor. He lost 65 pounds in weight (from 190 to 125 pounds) during the ensuing year, developed weakness and edema of the legs. He was unable to tolerate fats, spiced foods or sweets and numerous diets were tried without success. His condition at that time was diagnosed as duodenal ulcer and diffuse enteritis. After the onset of this illness he was hospitalized in four large hospitals in this area on many occasions. According to the transcript covering the last hospitalization in May 1944, "the patient had hydrothorax, ascites and massive edema of the legs which were relieved by multiple transfusions. He did well on a diet for a short time and then suffered a relapse. X-ray films showed some pylorospasm but no lesion in the esophagus, stomach or duodenum. One-half hour after ingestion of the barium, the head of the column reached the terminal ileum, indicating hypermotility. Some of the segments of the small bowel were distinctly narrowed and ribbon-like in appearance and there

* Received for publication, September 2, 1947.

were numerous discrete punched-out areas of filling defects in the jejunum and ileum. In addition, a portion of the small bowel was dilated. Barium enema showed filling of the entire colon except the fundus of the cecum which was somewhat spastic. The feces were clay colored, contained much fat, occult blood and a trace of bile. Glucose tolerance test revealed the following values: fasting, 70 mg.; 1 hour, 100 mg.; 2 hours, 68 mg. The blood phosphorus value was 3.3 mg., serum calcium, 9.8 mg. and the serum protein 5.6 gm. He was given intravenous injections of liver extract but this treatment had to be discontinued because of chills; and liver was then given orally." Five months before admittance to the hospital he developed thrombophlebitis of both legs which slowly improved.

Physical Findings

The physical findings at the time of his admission to this hospital were as follows: temperature, 98 F.; pulse rate, 88; respiratory rate, 20; blood pressure, 104/72; weight 120 pounds. The patient (Fig. 1) was intelligent and cooperative. He was emaciated, pale and chronically ill. The abdomen was slightly distended and diffusely tender, and palpation revealed peristaltic movements but no masses. There was general muscular atrophy of the extremities with moderate pitting edema of the legs extending up to the knees. Several small ecchymoses were visible on the legs and slight clubbing of the fingers and toes was present.

Laboratory Findings

Urinalysis, blood Kahn and Kline tests, sputum examinations for acid-fast bacilli, cephalin flocculation and Congo red tests were negative. Gastric analysis revealed fasting free hydrochloric acid 26 units, and total acid 38 units; after histamine, free acid 52 units and total acid 64 units. The hemoglobin content of the blood was 11.3 gm.; erythrocyte count, 4,390,000; hematocrit, 39; mean corpuscular volume, 88; mean corpuscular hemoglobin, 25; bleeding time, one and one-half minutes; clotting time, four and one-half minutes; leukocyte counts, from 3200 to 5500; differential counts, normal. Prothrombin time was fifteen seconds with a normal control of fifteen seconds; serum albumin levels varied from 1.3 to 1.4 gm., serum globulin from 2.1 to 2.9 gm. The feces were soft and brown; smears were negative for parasites, and cultures on four different dates were negative for acid-fast bacilli; Schmidt's qualitative test for urobilin was negative. The basal metabolic rate was normal. Bromsulfalein test (5 mg. per Kg.) showed 12 per cent retention of dye after forty-five minutes. The blood Vitamin C level was 0.6 mg. per 100 ml. The urinary urobilinogen was positive in a dilution of 1:80.

X-ray Findings

On November 24, 1944, films of the upper gastro-intestinal tract showed evidence of chronic regional ileitis (Fig. 2). On January 4, 1945, a barium enema revealed a normal appearance of the colon but decreased diameter of the terminal

ileum. Films of the chest were negative. Barium enema on February 21 (Fig. 3) showed an irregular constricted terminal ileum with suggestive evidence of ulceration and an irritable spastic cecum, indicating presence of terminal ileitis and cecitis.

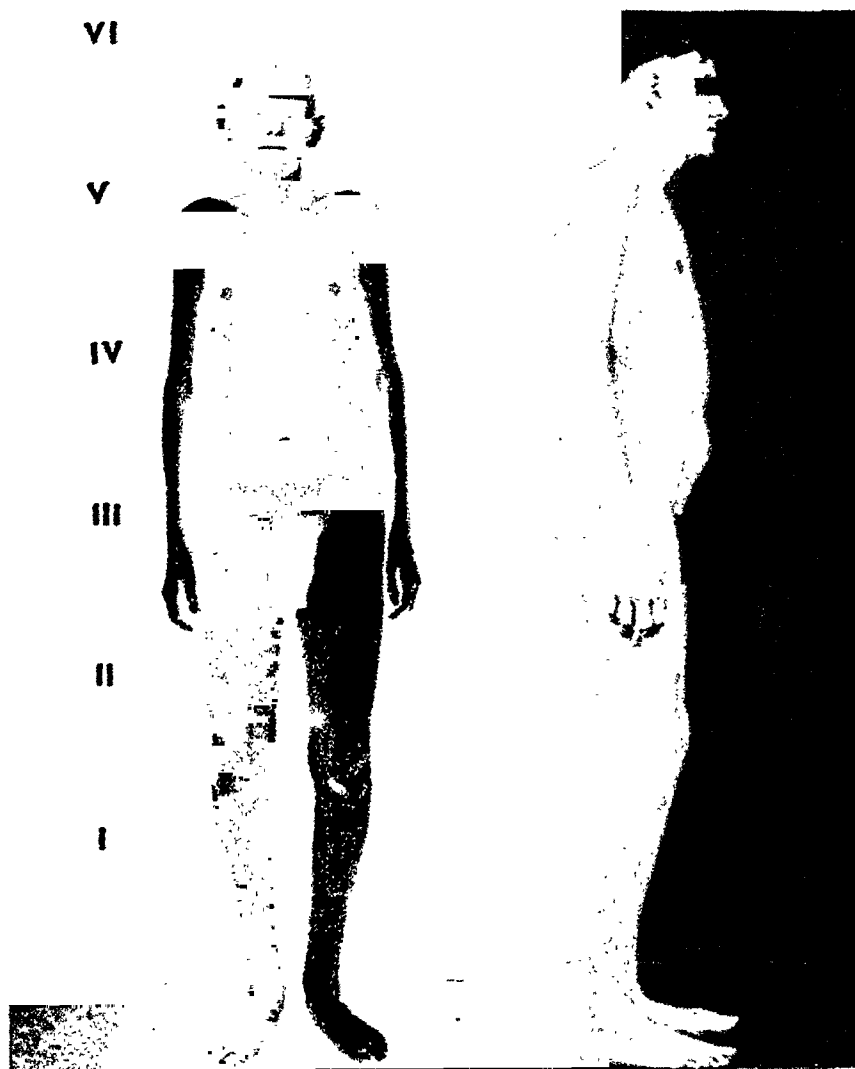


FIG. 1. Patient two months before date of expiration.

Hospital Course

He was placed on a diet rich in vitamins, carbohydrates and proteins and poor in fats, and was given water-soluble Vitamin K, liver extract and ferrous sulfate. Sigmoidoscopy on December 12, 1944 was negative. A tuberculin test (1:10,000) was negative. On December 22 and 23, he had a recurrent bout of diarrhea. On December 28, he developed ascites and edema of the trunk up to the level of the diaphragm, together with numbness and tingling of the fingers. He was given a series of transfusions of whole blood with slight improvement. During the week of January 8, 1945, edema of the legs and trunk increased and superficial

veins became apparent on the thighs and then on the chest wall. On January 19, the patient complained of abdominal cramps. On February 1, the liver was palpable at the costal margin for the first time. There was marked distention of veins over the chest and purpuric spots appeared on the legs. An ileotransverse colostomy and ileostomy were done on March 1. The terminal ileum was found to be thickened, edematous and dilated. A segment of ileum 1.8 cm. in length and 3 cm. in diameter, taken from the site of ileostomy and submitted for microscopic examination, showed submucosal and subserosal passive congestion, edema and round cell infiltration but no mucosal ulceration. The findings were considered compatible with an early stage of regional ileitis. The patient became distended on the following day and vomited 2000 cc. of dark fluid. A diagnosis was made of obstructive loop syndrome and a six-inch segment of small bowel was resected. On March 4, he became disoriented and showed marked pitting edema of the legs. On the next day he became comatose and the temperature rose to 104.6 F. Inflammation of the anterior abdominal wall developed and the patient expired on March 6, 1945, approximately sixteen weeks after admission.

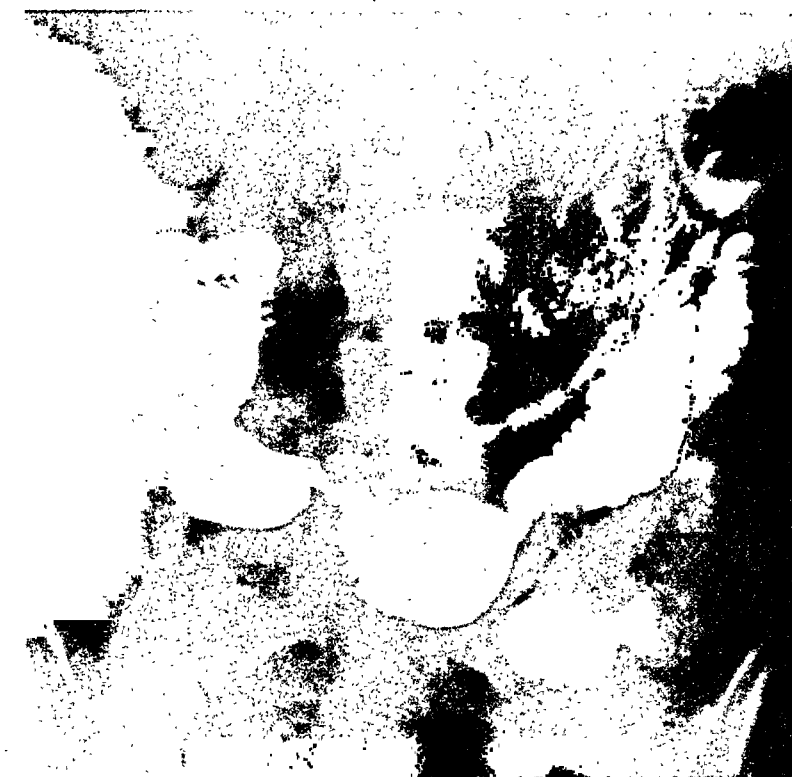
CLINICAL DISCUSSION

Dr. Norman R. Shippey, radiologist. "The deformity and narrowing of the ileum and contraction of the cecocolon which are well demonstrated by the x-ray films (Figs. 2 and 3) in this case are those of an ileocolitis. Tuberculosis, neoplasm, chronic ulcerative colitis, amebic colitis, sprue and regional or chronic cicatrizing enteritis must be considered in the differential diagnosis.

"One can tentatively rule out tuberculosis in that there is no evidence of this disease in the lung fields and active pulmonary tuberculosis would be expected in a lesion which is so extensive in the bowel. Neoplasm is usually associated with a much shorter history and would produce more sharply localizing signs, usually those of obstruction. Ulcerative colitis, to which ileitis may be secondary, usually produces a marked dilatation of the involved terminal ileum which may show a distorted mucosal pattern. The absence of typical lesions of ulcerative colitis in the rectum and sigmoid and the presence of lesions in the upper portion of the small bowel also do not support such a diagnosis. In amebiasis, the jejunum is not involved and parasites should be detected by examination of the stool. Steatorrhea and the primary deficiency diseases, including the condition known as sprue, can be ruled out because stenosis of the small bowel is not produced by these conditions. Without stenosis it may be difficult to differentiate these diseases by x-ray examination. Regional or chronic cicatrizing enteritis may involve any portion of the bowel from the jejunum to the sigmoid colon. This disease may produce any or all of the following roentgen signs:

FIG. 2. X-ray film two hours after barium meal showing irregular pattern of small bowel. Some loops show partial distension proximal to local areas of narrowing. There is evidence of terminal ileitis with small loop of distended bowel proximal to it.

FIG. 3. X-ray film after barium enema. The terminal portion of ileum and the cecum appear irregular and constricted and suggest the presence of ulceration.



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rapid or sluggish emptying of the bowel, segmentation, irregular filling, spastic contractures, variations in caliber of the intestine, increased fluid and gas content, narrowing of the lumen, rigidity of the intestinal wall and internal fistulous tracts. All of these signs, with the exception of a fistulous tract, were found in the present case, so that regional enteritis seems the most likely diagnosis. The changes in the small bowel are best demonstrated by interval films taken following ingestion of barium. A barium enema should always be carried out to determine the presence of involvement of colon and its extent, since there may be lesions in the colon which may extend down as far as the sigmoid.

"The presence of concomitant lesions in the small bowel and large bowel definitely points to the diagnosis of regional or chronic cicatrizing enteritis."

Dr. Charley J. Smyth, internist. "The presence of chronic diarrhea, periodic, crampy abdominal pain suspected at one time of being due to acute appendicitis, the low-grade fever, loss of weight and hypochromic anemia should have suggested the correct diagnosis in this case because these are the classical features of regional enteritis. This case was mistaken for nontropical sprue. The history of a poorly balanced diet and of the passage of large, frothy and very foul stools, and the finding of a flat glucose tolerance curve are expected in patients with nontropical sprue. In recent years there has been an increasing belief that the conditions referred to as sprue, idiopathic steatorrhea (nontropical sprue) and celiac disease of infants are similar, if not identical, diseases. At present, a mechanical obstruction to lymphatic channels or a chemical defect in fat absorption are the most acceptable theories of etiology of nontropical sprue. After x-ray study, nine days following admission, it was obvious that this patient had advanced regional enteritis. The clinician is dependent on the roentgenographic findings for the diagnosis of this disease.

"The question might arise as to why the diagnosis was not made sooner. The presence of tenderness in the epigastrium and the absence of tenderness in the lower part of the abdomen, particularly in the right lower quadrant, was an unusual feature in the case. A palpable abdominal mass is present in 50 per cent of cases and fistulas are commonly found in advanced stages of the disease. Neither of these findings was encountered in our patient. Perhaps the most important reason why attention was not focused on the small intestine as the site of the disease was the report that examinations of the small bowel and colon by x-ray methods in two other hospitals had shown a bowel pattern that is usually observed in sprue.

"Edema of the legs and trunk, which was mild and limited to the legs at the time of the first examination in this hospital extended, after seven weeks, to the trunk and finally to the lower portion of the chest. The persistent hypoalbuminemia of from 1.3 to 1.4 gm. per 100 ml. of blood was considered an adequate explanation for this finding and further possible causes for the extensive edema were not sought.

"In reported cases of regional enteritis in which the initial symptoms are those of partial intestinal obstruction, it has often been impossible to make a preoperative diagnosis of the cause of the obstruction. The decision to treat this man

surgically was based on his failure to respond to long continued oral and parenteral therapy."

Dr. Darréll A. Campbell, surgeon. "At the time of the patient's entry into this hospital, he showed advanced malnutrition as a result of his disease. His hospitalization fifteen years previously for suspected appendicitis is presumptive evidence that an inflammatory process was present in his ileum or cecum at that early date. This is important, for a decision regarding therapy may well be influenced by knowing whether the process is relatively recent or many years old.

"Clinically some authors have divided the disease into four groups: (a) acute, (b) irritative, (c) obstructive and (d) stage of fistula formation. It is doubtful if diagnosis is ever made in the first group since the roentgenologic signs and appearances at operation are still indefinite. The treatment in the remaining groups is probably surgical in the majority of instances, although some authors consider treatment, as in diverticulitis and peptic ulcer, to be primarily medical until complications arise that require surgical intervention. Pugh (Pugh, H. L.: *Ann. Surg.*, 122: 845-861, 1945) reported two cases in which the course of the disease was remarkably improved by the administration of penicillin. Medical treatment is important in preparing these patients for operation but, in itself, has not consistently produced satisfactory results.

"Patients who develop obstruction, internal or external fistulas, and probably also those whose condition continues to deteriorate despite conservative efforts, are probably best managed by some type of side-tracking operation with or without removal of the involved structures. It is to be expected that success usually will be attained after complete extirpation of the involved tissue in those patients in whom complications are minimal and whose general condition will tolerate such a procedure. Such successes have led some surgeons to recommend surgical removal of diseased tissue in all early cases. It has been shown, however, that recurrence in short segmental areas may follow such an operation. Moreover, involvement of the mesentery may have proceeded sufficiently far so that complete removal of the inflammatory process is technically difficult or impossible.

"In reviewing 164 cases, Garlock and Crohn (Garlock, J. H., and Crohn, B. B.: *J. A. M. A.*, 127: 205-208, 1945) found a mortality rate of 16 per cent in those patients who had primary resection, and recurrences in 19 per cent of the survivors. Such relatively high rates of mortality and recurrence ultimately led the Mt. Sinai group and others to employ a short-circuiting operation, such as ileotransverse colostomy or ileosigmoidostomy without resection of the inflammatory process. In 65 patients so treated, none died and the recurrence rate was 13.8 per cent. In patients in whom a second-stage operation had to be done for removal of the involved area, examination revealed almost complete healing in most cases. One of the chief proponents of complete extirpation of the involved area is Dixon of the Mayo Clinic, who reported unfavorable results with a simple short-circuiting operation. Two-stage resection has been discarded by Garlock and Crohn because (1) complete healing often follows the first stage, (2) it has an increased mortality rate and (3) recurrences are no less frequent

(36 per cent) than after the simpler first-stage operation. Surgical treatment must be performed in gentle stages in patients having complications, such as fistulas involving the small bowel, colon or bladder, while resection must be reserved for those patients in whom the physiologic function of the gastrointestinal tract is so altered as to make spontaneous recovery unlikely.

"This patient presented a far-advanced regional enteritis with such severe secondary nutritional changes, as to make it doubtful that he would have benefited from further conservative treatment. The operation, an ileotransverse colostomy, was the simplest one that might have been effective. In this operation, the distal portion of the ileum was brought out through a stab wound so that it might serve as a safety valve should obstruction occur at the ileocecal junction. It was realized, however, that he was an extremely poor operative risk."

POSTMORTEM FINDINGS

Principal Gross Findings

The body was of delicate asthenic build, the intercostal angle measured less than 90 degrees and the abdomen was scaphoid. The aorta was hypoplastic. There were evidences of wasting and the heart weighed 185 gm. Anasarca was present, with edema of the lower extremities and free fluid in all serous cavities. There was an old thrombus in the left popliteal vein and the thrombus extended upward to involve the left femoral vein, the left common iliac vein and the inferior vena cava to a point just proximal to the junction of the latter with the renal veins. Both renal veins showed recent thrombi. There were multiple recent pulmonary emboli without infarction.

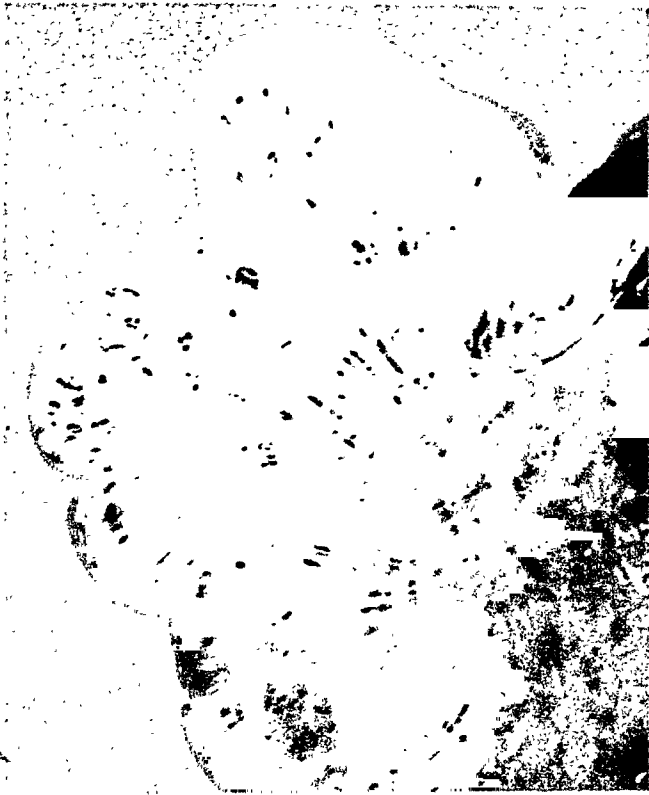
There was edema and superficial ulceration of the *stomach* with particles of clotted blood adhering to the mucosa. These findings were attributed to trauma from the tube which was used for suction of intestinal contents. The *duodenum* was moderately dilated. The *jejunum* was hyperemic and showed seven areas of rather sharply delimited constriction (Figs. 4, 5, 8, 9), the most proximal area being 20 cm. from the beginning of the jejunum and the most distal one, 60 cm. from the origin of the jejunum. Each constriction measured approximately 2 or 3 cm. in length, and the subserosa showed a varying degree of hyperemia. In several of the constricted areas the subserosa also showed grayish white nodules measuring up to 2 mm. in diameter. The *mesentery* corresponding to the affected portions of the jejunum contained large *lymph nodes* measuring up to 2 cm. in diameter which on section were white and apparently contained chronic purulent material. In the region between these

FIG. 4. External appearance of loop of jejunum showing segmental constriction with hyperemia and subserosal nodules.

FIG. 5. Loop of jejunum showing variation in caliber of lumen, several small ulcerations in mucosa of constricted upper portion, healing of mucosa of middle constricted portion, severe hyperemia of lower areas, relative uninvolved of intervening areas and enlarged mesenteric lymph nodes.

FIG. 6. External appearance of terminal portion of ileum.

FIG. 7. Terminal ileum showing extreme involvement of lower two-thirds with severe induration of walls, reduction of lumen and incomplete obstruction.



nodes and the line of attachment of the mesentery to the jejunum there were many pale white nodules measuring up to 2 mm. in diameter. On opening the constricted areas, there was irregular ulceration of the mucosa. In some segments only a portion of the circumference was involved; in other segments, the entire circumference was involved (Figs. 8, 9). In some areas, there was loss of continuity of the valvulae with superficial ulceration; in other areas with incomplete ulceration, there were small polypi (Fig. 9). The bases of the ulcers were red and ragged and the borders indistinct and slightly raised. In some areas which were less acutely hyperemic, the inflammatory processes appeared to be subsiding. The remainder of the jejunum and the ileum up to the point of ileo-transverse colostomy were moderately dilated, their mucosa in places somewhat hyperemic and their walls edematous. The terminal ileum from the point of ileal resection and up to its junction with the cecum measured 12 cm. in length and was dilated and saccular in the proximal 7 cm., where the wall varied from 3 to 14 mm. in thickness. The subserosa of the distal 5 cm. of the ileum (Fig. 6) was thickened, edematous, white and contained numerous white subserosal nodules measuring up to 3 mm. in diameter. This portion of the ileum was so constricted that there was difficulty in passing a probe through its lumen (Fig. 7). After opening this segment the circumference varied from 2.5 to 3 cm. and the wall from 1 to 2 cm. in thickness. The mucosa was grayish red in color, hyperplastic, chronically hyperemic and in some areas hemorrhagic (Fig. 7). The *mesoappendix* was chronically inflamed but the *appendix* was free of any lesion. The *cecum* was shortened (Fig. 10), measuring 6 cm. in length, was severely deformed and had a maximum circumference of 6.5 cm. and a narrowed lumen. The mucosa was chronically hyperemic, markedly hyperplastic and irregularly ulcerated. The walls were greatly thickened. The regional *lymph nodes* of this area were greatly enlarged measuring up to 1.5 cm. in diameter and were white and necrotic in appearance. The *ascending colon* up to the point of anastomosis presented a varying amount of fibrosis, a number of chronic miliary abscesses and severe thickening of the walls. The remainder of the large bowel was grossly uninvolved. There were no fistulas.

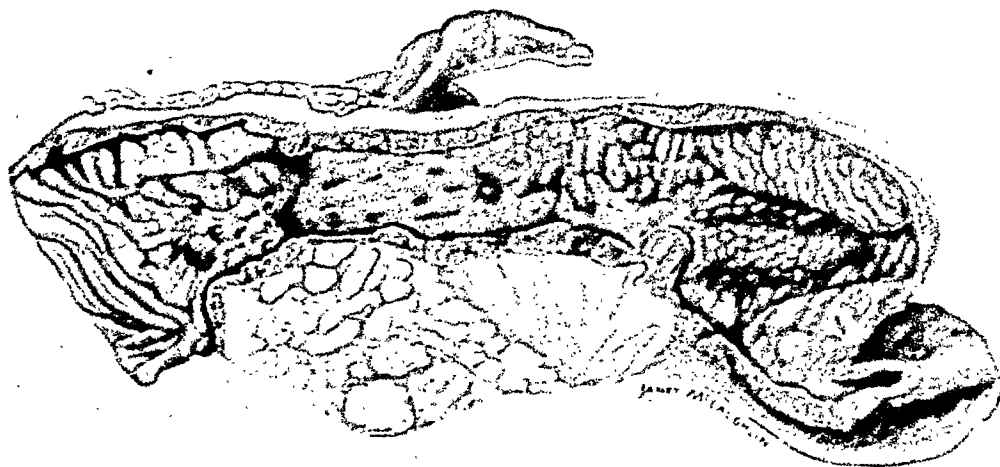
Microscopic Findings

Sections of the *stomach* showed chronic ulcerative gastritis, with blood pigment adhering to the surface and chronic edema of submucosa with lymphatic obstruction (Fig. 11). Sections of the *jejunum* (Figs. 12-14) showed a vascular chronic granuloma with infiltration extending to all layers, including the subserosa, but being most marked in the mucosa and submucosa. In some areas,

FIG. 8. Drawing of loop of jejunum showing segments in various stages. The lesions at the upper left are recent and relatively acute, that at the lower end, subacute. Note nodules in mesentery and a large lymph node which contained a chronic abscess.

FIG. 9. Drawing of a loop of jejunum showing subacute ulceration and nodular granulomatous inflammation.

FIG. 10. Drawing of cecum showing marked contraction of lumen, thickening and fibrosis of walls, old ulceration and extensive inflammation. Note the many large whitish mesenteric lymph nodes. These showed chronic abscess formation and granulomatous reaction.



there was fibrosis, increase in capillaries and prominence of arterioles. In places, the myenteric plexus was prominent and about it there was chronic inflammation. The *mesentery* of the jejunum showed severe chronic lymphadenitis (Fig. 15) and some fibrosis and there were thrombi of fibrinoid material in dilated lymph vessels. Stains for amyloid were negative. Sections of mesentery revealed localized necrotizing lesions, chronic abscess formation and nodular granulomatous lesions (Fig. 17). In the *lymph nodes* there was hyperplasia of follicles and of endothelial cells, areas of plasma cell infiltration and occasional multinucleated giant cells. The *mesoappendix* was chronically inflamed but the *appendix* itself was relatively uninvolved. One section of *ascending colon* showed chronic submucosal and subserosal edema and inflammation and a subserosal abscess, and in one section of rectum there was ulceration, chronic granulomatous reaction of the mucosa and submucosa and chronic edema of the submucosa. Sections of *liver* (Fig. 18), *spleen*, *adrenals*, *lungs* and *mesenteric lymph nodes* showed multiple focal granulomatous lesions. A number of these focal lesions, particularly those of submiliary size, revealed caseation necrosis. All of these focal lesions exhibited epithelioid cell reaction and most of them foreign body giant cells. The giant cells were located both centrally and peripherally; their nuclei were not always arranged in horseshoe pattern and in the cytoplasm of some of the cells there were visible oval vacuoles or refractile foreign particles. All these minute focal lesions were considered tuberculous. Two isolated acid-fast bacilli were found in a stained section of a mesenteric lymph node. Occasional acid-fast bacilli were also found in smears from another mesenteric lymph node and from a jejunal ulcer, in both instances after fixation of the gross specimen. The bacilli had the morphologic characteristics of tubercle bacilli. A number of sections from representative lesions in the ileum and colon were stained for acid-fast bacilli with negative findings. The brain showed no lesion.

Final Anatomic Diagnoses

The final anatomic diagnoses were: segmental enteritis involving jejunum, terminal ileum, cecum and ascending colon; oldest lesions in cecum and terminal ileum; incomplete obstruction in ileocecal region; chronic suppuration, abscess formation and granulomatous inflammation in affected portions of bowel and respective portions of mesentery and mesenteric lymph nodes; chronic edema of submucosa of stomach; chronic ulcerative gastritis (traumatic); small ulceration with granulomatous inflammation of rectum and chronic edema of submucosa; thrombosis of deep veins of left lower extremity; thrombosis of left popliteal and iliac veins with varying degree of organization and canalization; recent thrombus in inferior vena cava with beginning organization; recent thrombi in renal veins

FIG. 11. Submucosa of stomach showing chronic edema and fibrinoid material in lymphatic spaces. $\times 85$.

FIG. 12. Submucosa of jejunum showing portion of a small abscess. $\times 85$.

FIG. 13. Small abscess of submucosa of jejunum extending into inner muscular layer. $\times 85$.

FIG. 14. Mucosa and submucosa of jejunum showing healing by fibrosis. $\times 85$.

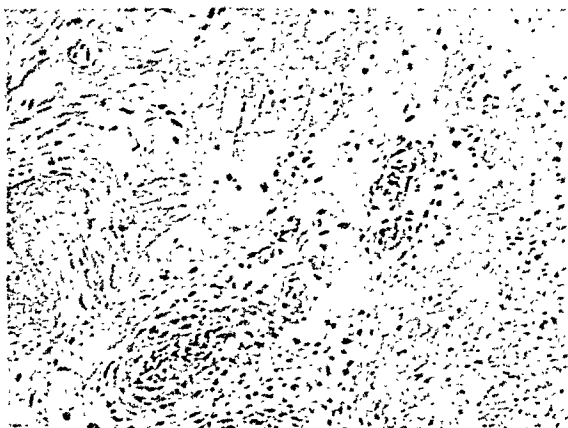
FIG. 15. Subserosa of mesentery of jejunum showing chronic inflammation. $\times 85$.

FIG. 16. Subserosa of terminal ileum showing portion of a granulomatous nodule. $\times 85$.

FIG. 17. Mesenteric nodule showing subacute granulomatous reaction suggesting early healing stage. $\times 70$.

FIG. 18. Tuberculoid focus in liver. Note inclusions in giant cells. $\times 85$.

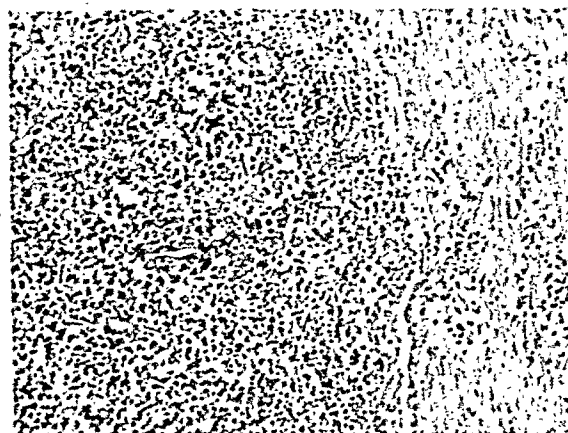
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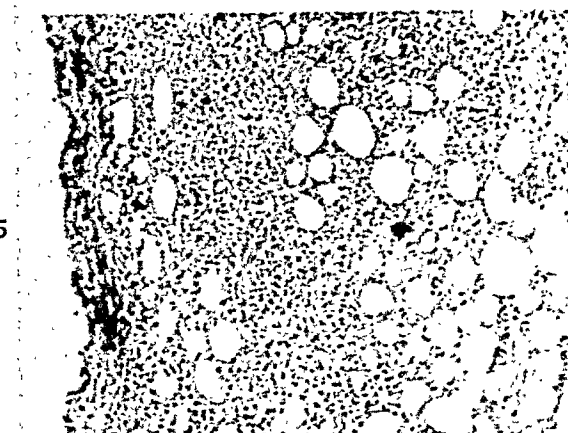
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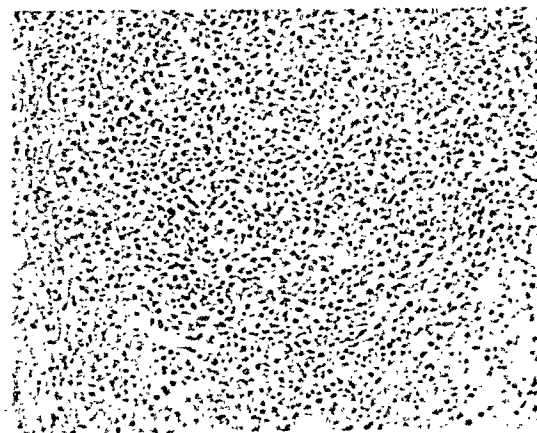
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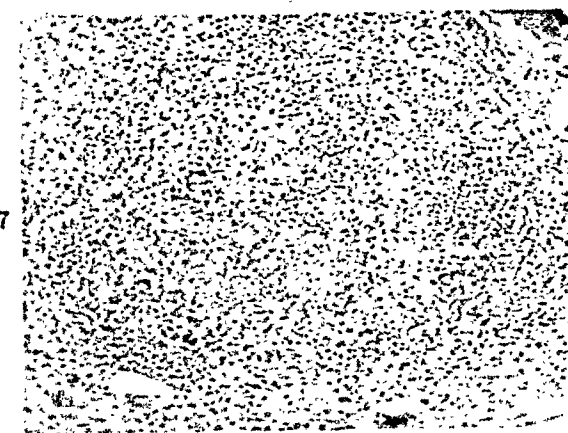
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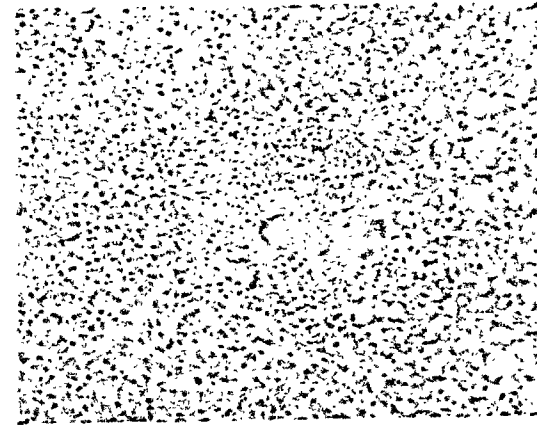
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and ileocolic branches of the superior mesenteric vein; multiple recent pulmonary embolism; purulent bronchitis and beginning bronchopneumonia; moderate fatty metamorphosis of liver; atrophy of heart; hypoplasia of aorta; hypoplasia and atrophy of testes; chronic pyelonephritis; miliary and submiliary tubercles with caseation in liver, spleen, lungs, adrenals and mesenteric lymph node.

CLINICOPATHOLOGIC CORRELATION

The disturbances in nutrition, resulting from ulceration of the bowel and the interference with intestinal absorption were probably responsible for the hypochromic anemia and the lowered values for serum albumin and total protein. The nutritional disturbance also explains the low glucose tolerance and the low Vitamin C level. The chronic passive congestion of the liver together with the fatty metamorphosis and the focal lesions in that organ explain the mild degree of bromsulfalein retention, the increased urine urobilinogen and the palpable liver. The superficial veins on the chest must be related to the development of thrombi in the inferior vena cava.

It has been pointed out that patients with regional enteritis usually are of nervous excitable disposition which is also true of patients who are prone to develop peptic ulcer or chronic ulcerative colitis. It is probable that some segments of bowel undergo complete healing and evidence for such healing was present in our patient. If ulceration is severe or extensive, however, the lesion may very well become chronic and undergo fibrosis and contraction. In the latter case, surgical removal would seem justifiable.

A striking feature of this case is the long history of intestinal symptoms from the age of three years, an acute exacerbation at the age of thirteen and continual active chronic disease for the last five years of life. Pathologically there were old chronic active lesions in the ileum and cecum and acute, subacute and apparently healed lesions in the jejunum. It is interesting to note that during the last period of hospitalization the patient had no tenderness in the right lower quadrant in the region of the old chronic fibrosing lesions, but that he did complain of tenderness in the epigastrium which is to be related to recent active lesions in the jejunum.

Acid-fast bacilli, when found in regional enteritis, have usually been attributed to a complicating tuberculous infection. The presence of a few acid-fast bacilli in smears of two lesions at autopsy and in a microscopic section of another lesion, plus the many miliary and submiliary granulomatous nodules, with and without caseation necrosis, point to the presence of tuberculosis. Cultures of stool for tubercle bacilli during life revealed no growth. Hadfield (*Lancet*, 2: 773-775, 1939) found noncaseating giant cell systems in 13 of 20 patients with regional enteritis. He stated that the giant cells are not typical of those found in tuberculosis. Instead of the tendency for such giant cells to be disposed peripherally in the lesion and to have a horseshoe arrangement of the nuclei, the giant cells in enteritis are often centrally placed, have numerous nuclei which sometimes largely fill the cell and the cytoplasm contains refractile bodies or other ingested foreign material. While miliary and submiliary granulomatous lesions in the lung, liver, spleen, adrenals and mesenteric lymph nodes corre-

sponded to the giant cell systems described by Hadfield, there were others with definite caseation, particularly in the spleen, liver and lymph nodes, and all of these lesions were considered to be tuberculous. Even in the areas of caseation, some of the foreign body giant cells were atypical for tuberculosis and contained visible particulate matter. Because of the size and appearance of these focal lesions and because of their occurrence in the spleen, they were probably terminal phenomena spread by way of the blood. A primary focus of tuberculosis was not found in the lung.

The diagnosis of regional enteritis implies that this is a disease of unknown etiology and that in the present instance the terminal miliary tuberculosis was a complication not directly related to the enteritis. It is, however, possible that the entire picture is one of chronic tuberculous enteritis with secondary infection, in which the behavior of the tubercle bacillus or the reaction of the patient to the bacillus is atypical. In the relationship of regional ileitis to tuberculosis there are many parallels to the relationship of Boeck's sarcoid to tuberculosis. These include the negative tuberculin reaction in high dilutions, the chronic course, the atypical granulomatous lesions and the occasional finding of caseation necrosis and tubercle bacilli in the lesions.

EDITORIALS

RICKETTSIALPOX, A NEW DISEASE

Rickettsialpox is a recently discovered rickettsial disease occurring exclusively, so far as is known, among human beings and mice in New York City. Primarily a murine disease transmitted by the rodent mite *Allodermanyssus sanguineus*, rickettsialpox produces a unique nonfatal febrile illness in man. Clinical features in the order of their appearance consist of an initial lesion (presumably at the site of a mite bite), regional lymphadenopathy, a spiking fever, and a generalized varicelliform rash. Leukopenia represents the only positive finding of routine laboratory examination. Bacterial agglutinations including the Weil-Felix test are uniformly negative. The employment of yolk sac preparations of *Rickettsia akari*, the causative agent of rickettsialpox, as antigen in the complement-fixation test provides a useful tool in laboratory diagnosis. As in other rickettsial diseases, the test remains negative until late in the second week after onset of fever. The titer reaches its peak during the third or fourth week, following which it falls slowly. A detectable titer, however, may persist for more than a year after the attack. The rickettsia of rickettsialpox has been found to be immunologically related to the rickettsia of Rocky Mountain spotted fever. Although not identical, stock antigens prepared from both organisms show considerable serologic similarity. This creates a practical difficulty, from a laboratory standpoint, in the differentiation of rickettsialpox and Rocky Mountain spotted fever. This difficulty is somewhat minimized by wide clinical and epidemiologic differences as well as by the fact that the majority of Rocky Mountain spotted fever patients give a significant titer with Proteus OX19. Borderline cases will occur, however, and specific antigens which can be produced in quantity represent highly important objectives.

The appearance of a new disease in the most populous of modern cities raises a number of pertinent questions, the most important of which relate to the origin and distribution of the disease and especially to methods of control. There are no clues available at this time concerning the origin of rickettsialpox and, since it has not been described elsewhere, its distribution outside New York City is unknown. The distribution of the several hundred confirmed cases of this domiciliary disease in New York City, however, would appear to postulate a widely disseminated involvement of rodent colonies. Nearly one hundred foci of infection have been incriminated in four boroughs of the city. Under these circumstances it is difficult to see how the disease could be limited to New York City alone. The records of New York physicians indicate that the illness has been occurring at some of the foci for at least four years; however, the distribution of the cases indicates that a much longer period of time would be required for so extensive an involvement of rodent colonies.

The rodent mite, *Allodermanyssus sanguineus*, was described by Hirst at the beginning of the century as a parasite of rats in Egypt. Since then it has been reported from a number of cities in this country, notably Boston, Philadelphia and Washington, D. C. Its occurrence, however, until recently was regarded

by entomologists as somewhat of a novelty. Very little is known about the habits and physiology of this mite.

The control of rickettsialpox, which promises to be a difficult task, depends chiefly upon identification and eradication of infected mouse colonies. The mites appear to be completely dependent for survival upon a rodent host. Serologic surveys of mouse populations should aid considerably in such an undertaking since serums of mice trapped at foci of infection show a high incidence of positive reactions, a phenomenon not shared by serums of laboratory mice and of mice collected in a limited survey conducted in Northern Virginia.

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ROBERT J. HUEBNER

REGIONAL ENTERITIS

When unique inflammatory lesions involving the terminal portion of the ileum were first described by Crohn and his associates in 1932 (Crohn, B. B., Ginsburg, L., and Oppenheimer, G. D.: J. A. M. A., 99: 1323-1329, 1932), the discovery of the etiology of this entity became a challenge to clinicians and epidemiologists. Was this a new disease which suddenly manifested itself in different parts of the globe as a subendemic enteritis? Crohn's classic paper put us far along the road to proper evaluation, but the sporadic case reports which followed failed to advance greatly our knowledge or further our understanding of this complex disease. Sproull, in 1936, summarized the known facts on 123 cases, correlating the established points in etiology, pathology and clinical findings (Sproull, J.: Am. J. Roentgenol., 36: 910-920, 1936). Advances in clinical interpretation and roentgenographic findings followed fairly rapidly during the following years.

Some considerable confusion has existed in regard to nomenclature. For several years the disease was known under the name of *terminal ileitis* as it was thought to be confined to the terminal ileum. It then became apparent that this unique process might affect any part of the intestines and involve not only the small bowel, but also the colon, especially the cecum, and that it was probably associated with the long known, but little understood, condition of *ulcerative colitis*.

At first, the clinical and roentgenographic aspects of the disease overshadowed the knowledge of the pathology of this condition, owing mainly to conservative treatment by the surgeon, his reluctance to obtain material for biopsy and the fact that no one clinic had sufficient data upon which to make adequate studies. During the past few years, however, more knowledge has been gained of the histopathology and the gross pathologic anatomy of this entity. Mixer's review in 1939 did much to clarify the different stages of the disease (Mixer, C. G.: Ann. Surg., 102: 674-682, 1939). Three clinical phases have been delineated. The first or acute phase is characterized by diarrhea, tenesmus, fever and signs simulating those vaguely classed in the category of "the acute abdomen". The second clinical phase begins when stenosis supervenes, and the symptoms are those of intestinal obstruction. The terminal phase is one of fistula formation and may be so complicated as to mask the underlying condition.

The pathology of *regional enteritis* has been well reviewed by Owens (Owens, F. M.: Arch. Surg., 48: 465-471, 1944) and Schepers of Johannesburg, South Africa (Schepers, G. W. H.: Am. J. D. D., 12: 99-116, 1945). Both authors stressed the broad conception of the disease, emphasizing the "skip areas", the red "rubber hose" appearance of the bowel, and the probable association with ulcerative colitis. Owens described neuromuscular hyperplasia as a characteristic feature but hesitated in ascribing its etiology. Schepers emphasized the small serosal granulomas which are characteristic of the disease, sometimes simulating sarcoid. He divided the disease into two components:

1. A primary phase characterized by:

(a) A stage of edema of the submucosa and serosa with dilatation of submucosal lymphatics and hyperemia of the juxtamuscular adventitial blood vessels.

(b) A stage of plasma cell infiltration of the submucosa and serosa,

(c) Diffuse fibrosis, with disappearance of the plasma cells, except where the latter are trapped,

(d) Healing; and

2. A secondary phase, characterized by ulceration superimposed on any of these phases of the primary lesion, with corresponding modification of the pathologic process. There are tendencies to early or late perforation, fistulation and granuloma formation.

In evaluating the etiology of this condition, one is impressed by two prominent features, namely: the hyperplasia of the neuromuscular elements with signs of irritation of Meissner's plexus or its central connections; and the usual presence of a heavy eosinophilic cellular infiltration. Along these two lines, two theories of causation have evolved, one concerned with a neuropathy, the other with an allergy. Although the lesion is of manifest inflammatory nature, the part played by many bacterial agents is probably secondary. Nonmotile *Escherichia coli*, *Streptococcus viridans* and other organisms have been recovered from many lesions but no conclusions have been drawn as to their relationship to the lesions. Because of the granulomatous nature of the disease, search has been made for acid-fast organisms by direct staining, culture and guinea pig inoculation, but has proved fruitless. The similarity to Boeck's sarcoidosis has brought up the association of this condition with regional enteritis, but so far no conclusive evidence of a similar causation has been established. There may be some remote association with lipophagic granulomatosis of the enteric tract.

With the increasing frequency and recognition of this condition, pathologists are in an excellent position to add much to the knowledge of this confusing disorder, both as to etiology and pathology of its many complex stages.

Oakland, California

PAUL MICHAEL

CONCERNING METHODS OF TISSUE STAINING

The importance of staining methods in clinical pathology, in both its diagnostic and investigative aspects, can hardly be overestimated. The rapid progress made in normal and pathologic histology would be unimaginable without the aid of steadily improving staining techniques which permit the differentiation

of otherwise indistinguishable histologic details. In the beginning, staining methods were devised on a purely empirical basis. The only aim was to impart different shades to various morphologic structures. Today, however, there is an increasing tendency to make color contrasts meaningful in terms of tissue chemistry. There is good reason to believe that in the future histochemical techniques will contribute considerably to our understanding of the functions of the cells and tissues.

The number of new staining techniques and of modifications of old ones published yearly is so large that it is impossible for the practical clinical pathologist to keep pace with them. Fortunately, the simplest stains will permit accurate diagnosis in the vast majority of cases. This fact, together with lack of time and of expert technical aid, possibly accounts for the routine exclusive use of hematoxylin and eosin, after formalin fixation, by a very large number of pathologists. However, for purposes of research in pathology, sometimes even for those of diagnosis, this simple technic will often fall short of giving the desired information.

The files of slides and of tissue blocks of the clinical pathologists form a real treasury to draw from whenever old cases have to be reexamined and reevaluated in the light of new knowledge. Quite often such reexamination involves the application of exacting staining techniques to the old material. How disappointing it is to find that the precious material is utterly wasted, as far as science is concerned, because it has been treated in such a way that finer staining methods are impossible. It is so easy to prevent this waste. Most of the irreparable damage is done by improper fixation. The most common cause for improper fixation is the use of pieces of tissue which are too thick. No fixing fluid can penetrate any tissue block thicker than about 3 mm. fast enough to prevent autolysis in the center of the block. The next important point is the choice of the right fixative. Formalin does not yield entirely satisfactory results with many of the more delicate methods. Probably the best universal fixative for practically all staining methods, with the exception of those for the demonstration of enzymes, is Bouin's fluid, especially that with the acetic acid strength reduced to 2 per cent, or Masson's modification (0.1 to 0.5 per cent trichloroacetic acid instead of acetic acid). This fluid can be made up in bulk and will keep indefinitely. For enzymatic stains, fixation in "chilled acetone" is required. For certain special purposes (as neurohistology and demonstration of Golgi apparatus and mitochondria), the use of other fixatives may be indicated. The third point is careful dehydration. Tissue blocks carried through the alcohol series in a rush often are incompletely dehydrated and suffer marked shrinkage in the course of embedding. The blocks should be embedded in paraffin in which they remain unchanged indefinitely. The dilute alcohol in which celloidin blocks are stored ruins the tissues within a few weeks.

This editorial ends with the following plea to all clinical pathologists: Besides running your tissues for diagnosis in your routine way, save, whenever the amount of tissue available permits, one Bouin-fixed and, for enzymatic stains, one acetone-fixed paraffin block from every case. The amount of extra work involved is minimal, and the returns will be gratifying.

Chicago

GEORGE GOMORI

SELECTED ABSTRACTS

Cytologic Recognition of Cancer in Exfoliated Material from Various Sources; Useful Modifications of the Papanicolaou Technique. WARREN C. HUNTER AND HOWARD L. RICHARDSON. Surg., Gynec. and Obst., 85: 275-280, 1947.

The authors offer certain modifications of the method originally described by Papanicolaou for the cytologic recognition of carcinoma in exfoliated material. The following procedure is carried out in the preparation of specimens of bronchial aspirations, gastric fluid, rectal mucus, ascitic or other fluid and urine. Those obtaining the specimen add immediately a good quantity of 10 per cent formalin which serves to arrest autolysis and begins the process of fixation. When the material reaches the laboratory the technician adds a quantity of saturated aqueous solution of picric acid which is a good protein precipitant and also a good fixative. The material is then filtered through filter paper and the residue is pressed into the tip of the cone with a rod until it becomes a compact ball. It is then imbedded in paraffin and the sections stained by the Papanicolaou technic.

The authors found that this method has many advantages: (a) cells of all kinds are well preserved; (b) the cells are concentrated, obviating the necessity of long search over wide areas; (c) the cells are all in the same focal plane and do not overlie one another; (d) a number of sections can be cut and mounted on a single slide, assuring adequate sampling and affording economy of slides; (e) groups of cells are not torn apart as with smearing, thus enabling one to see cells in a pattern and in their relation to one another.

In preparing vaginal fluid the authors adhere to the original Papanicolaou technic of making a smear at the time the material is obtained and of fixing it immediately in alcohol-ether. The remainder of the specimen is not discarded, however, but is fixed in 70 per cent alcohol, imbedded and the sections stained by the Papanicolaou method. The latter was found to be a valuable adjunct to the vaginal smear examination.

Using this procedure, Hunter and Richardson were able to give valuable aid to the bronchoscopists and, on a number of occasions, were able to make a positive diagnosis of carcinoma in the presence of a negative biopsy. The prompt addition of formalin or picric acid to material from gastric aspirations prevented digestion so that normal gastric mucosa and neoplastic cells could readily be identified. In the examination of urine specimens it was found that the cytologic differences between normal mucosa and carcinoma here is as clear-cut as in other locations. The authors also believe that this method will be valuable for detecting colonic cancer from specimens of rectal mucus. In examining transudates and exudates it was found that much time and effort can be saved by the simple procedure of precipitating protein from the fluid with picric acid, filtration, imbedding and sectioning.

The value of the Papanicolaou stain was emphasized. The authors considered it superior to other stains because of the striking sharpness with which the nuclei stand out under low power and the fine nuclear detail brought out by higher magnification.

Detroit

O. A. BRINES

A Classification of Ovarian Tumors Based Upon Histogenesis. JOHN A. SPENCER AND PHILIP J. REEL. Am. J. Obst. and Gynec., 54: 273-280, 1947.

The authors have proposed a classification of ovarian tumors based on histogenesis, which is similar to that suggested by Schiller. There are three main groups with several subdivisions under each.

Group I is designated as Ovario-genetic and includes tumors developed from the tissues of true ovarian structure. Specifically they are the granulosa cell and theca cell tumors, arrhenoblastoma, dysgerminoma and fibroma, angioma and myoma. The cystomas and endometrial tumors are here classified.

Group II is designated as non-ovario-genetic and includes tumors developed from tissues not normally present in the ovary but which have been included in the ovary in fetal life, either by displacement of totipotential tissue or by displacement of cell rests from nearby

structures. In this group are placed the metastatic tumors of the ovary which develop during adult life. The members of this group are teratoma, hypernephroma, mesonephroma, Brenner tumor, ganglioneuroma and Krukenberg's tumor.

Group III includes malignant tumors of undetermined origin and it is suggested that these represent almost completely undifferentiated forms of tumors which, if they were better differentiated, could be recognized as tumors in Group I or II. The carcinoma simplex and the scirrhous and plexiform carcinoma find their place in this group.

The classification is logical, complete and practical and will be useful for pathologists who encounter these tumors.

O. A. BRINES

Gastric Acidity Before and After Development of Carcinoma of Stomach. M. W. COMFORT and M. P. KELSEY. *J. Nat. Cancer Inst.*, 7: 367-377, 1947.

Gastric analyses were performed in 277 patients in whom a diagnosis of gastric carcinoma was subsequently established. There was a period of from 2 to 30 years (average 11 years) prior to the development of the cancer. This study revealed achlorhydria in 127 patients (45.8 per cent), a figure significantly higher (by 24 per cent) than that obtained in control groups of the same ages and sex. Also, the group of patients with apparently intact acid secretion prior to the detection of cancer exhibited lower values of free acid than the control series, if patients suffering from peptic ulcer at the time were excluded. Finally, comparison between the acid values observed years before, and those found after the development of cancer showed a progressive drop in most instances.

The authors believe that the most likely interpretation of these findings is the assumption of a prevalence of chronic atrophic gastritis, leading to progressive destruction of acid-secreting cells, in the patients who subsequently developed gastric carcinoma.

Chicago

KURT STERN

Preservation of Sheep Red Cells for Complement-Fixation Tests. J. PORTNOY, N. H. BOSSA and A. HARRIS. *Ven. Dis. Inform.*, 28: 137-141, 1947.

Seven methods used for the preservation of sheep blood are compared. Two methods were found to be superior and to be equally satisfactory. The authors, modifying the Ashby technic, mixed twelve volumes of a 3.5 per cent solution of sodium citrate with ten volumes of blood. In addition, it was found that the keeping qualities of sheep blood were dependent upon the quantity of available oxygen. Hydrogen peroxide (6 parts of a 3 per cent solution per 100 parts blood mixture) provided a readily available oxygen source.

Alcohol as a Disinfectant Against the Tubercle Bacillus. C. R. SMITH. *Pub. Health Rep.*, 62: 1285-1295, 1947.

Alcohol is an effective disinfectant against tubercle bacilli in the wet and dry states. While a 70 per cent solution is the most effective single strength, a 50 per cent solution is best for dry states and a 95 per cent solution is best for wet states. Tubercle bacilli in smears dried from sputum are usually killed by 50 to 70 per cent ethyl or 30 to 80 per cent isopropyl alcohol in one to two minutes; in very thick smears the bacilli survive the action of 70 per cent ethyl alcohol for five minutes, but are killed within ten minutes.

Influence of Penicillin on the Coagulation of Blood. ALEXANDER FLEMING and E. W. FISH. *Brit. M. J.*, No. 4519: 242-243, 1947.

The local application of penicillin in concentrated form, as in powder, was shown to have a retarding action on coagulation and on contraction of the clot. The local use of penicillin in an operation where the formation of a firm blood clot is of importance, as in dental surgery, should be limited to washing out the cavity with a solution of the drug in a concentration not exceeding 100 units per ml. These results have no bearing on the systemic application of penicillin.

BOOK REVIEWS

Rh . . . Its Relation to Congenital Hemolytic Disease and to Intragroup Transfusion Reactions. EDITH L. POTTER, M.D., Ph.D., Assistant Professor of Pathology, Department of Obstetrics and Gynecology, The University of Chicago and the Chicago Lying-In Hospital. 344 pp., 65 figs., 17 tables. \$7.50. Chicago: The Year Book Publishers, 1947.

This comprehensive monograph is the second publication on the clinically important subject of isoimmunization by the Rh factor, Broman's review having been published in 1944 at Stockholm. It is not a simple task to cover all aspects of this subject which border on the fields of pathology, pediatrics, hematology, biochemistry, serology and genetics. By and large, Potter has done a commendable job in assembling the vast material, much of which is not sound scientifically. It is to her credit that she has not been misled by claims which have been recently made in explanation of some of the manifestations of Rh incompatibility, *e.g.*, (X proteins, conglutination, pathogenesis of kernicterus, clinical correlation of symptoms depending upon the presence of agglutinins or blocking antibodies, and congenital malformations induced by isoimmunization).

The first four chapters cover the historical background of individual blood differences, the discovery of the new blood factor and the series of events which led to the serologic and genetic complexity of the Rh factor. The book was written just about the time that American workers were gradually abandoning the multiple allelic theory in favor of the Fisher-Race theory of linkage at three different loci. The reviewer cannot accept Potter's suggestion that "a change in terminology would not necessarily be involved". The reader will avoid the utter confusion created by the older terminologies of the multiple gene theory, if he first studies the much simpler Fisher-Race theory which takes into account the three main Rh factors (C, D and E) and their genetically related Hr factors (c, d and e).

The reviewer must take exception to Potter's stand on the mechanism of transplacental isoimmunization. It is highly probable that early abortions, and complications of labor do not by themselves play the essential rôle in transferring fetal blood into the maternal circulation. Since all workers are agreed that minute quantities of fetal blood are sufficient for isoimmunization, such passage may occur during the course of each and every normal pregnancy. In this connection the occurrence of erythroblastosis fetalis in the first born is important, independent of the influence of previous intravenous or intramuscular injections of blood. Incidentally, the author does not sufficiently appreciate the antigenic properties of blood administered either by subcutaneous or intramuscular route.

Potter contributes a service in taking a stand that, aside from the rôle of the Rh factor in erythroblastosis fetalis and transfusion accidents, the Rh factor is not related to abnormalities of pregnancy or other conditions of the fetus or newborn infant. The several claims regarding congenital malformations, abortions and premature separation of the placenta are not supported by statistical studies.

The author's long experience with newborn infants is reflected in the excellent sections on the clinical and pathologic features of erythroblastosis fetalis and the differential diagnosis. There are numerous illustrative photographs of the gross and microscopic pathology. In the section on treatment of the affected infant with transfusion of Rh-negative blood, no mention is made of the precipitin test for the differentiation of those infants who require intensive therapy and perhaps a replacement transfusion, from those who will perhaps require one or two transfusions. The test is, however, referred to elsewhere in the text. Recent experience with the study of the cord blood with the precipitin test of Coombs, Mourant, and Race reveals that it is far more sensitive than other tests. The reviewer can only applaud her view that exsanguination transfusion should be limited to carefully selected cases. Potter offers the excellent suggestion that the circulation through the cord of affected infants should be interrupted immediately after delivery.

In the last chapter which deals with technical procedures there are several statements

which may be challenged. In large scale work it is neither feasible nor desirable to use a separate pipet for each blood to be tested. At this writing there is no experimental serum of animal origin which will meet the criteria laid down by the National Institute of Health, *i.e.*, 32 titratable activity units. Although the blood of the fetus will frequently contain factors C or E in addition to D, the mother will more often produce only anti-D (anti-Rho) because D is far more antigenic than either C or E.

The enormity of Dr. Potter's task is illustrated by the 794 complete references containing remarkably few established facts which are lost in a veritable maze of unsubstantiated claims and numerous papers which contribute nothing but confirmation. This monograph should be of considerable help to the reader who may otherwise float in a sea of doubt.

Raritan, New Jersey

PHILIP LEVINE

Encyclopedia of Endocrinology. Section IV, Volume VII, in two independent units: (1) Ovarian Tumors, (2) References. By HANS SELYE, M.D., Ph.D., D.Sc., F.R.S., Director of the Institute of Experimental Medicine and Surgery, University of Montreal. 776 pp., 37 plates. \$21.75. Montreal, Canada: Richardson, Bond and Wright, 1946.

This comprehensive work on Ovarian Tumors appears in loose leaf form contained in inflexible three-ring leather covers. The unit on Ovarian Tumors contains 289 pages with 60 additional pages of periodical references. There are 37 full page plates with 5 to 9 illustrations on each, including photographs of patients, gross specimens, photomicrographs and x-ray photographs. The subject matter is indexed and divided into sections by subject dividers. The reference unit contains 427 pages of bibliography. There are 15,000 references on ovarian tumors.

In the unit on Ovarian Tumors, the author comments that the main purpose of the Encyclopedia "is to demonstrate, using endocrinology as an example, the essential feasibility of a rational classification and evaluation of all publications pertinent to a large field of medicine". The volume by no means is confined to discussion of endocrinology but portrays all aspects of ovarian tumors in such a manner that the text will have wide appeal to gynecologists, endocrinologists and pathologists.

The text begins with a "Discussion of Ovarian Tumors in General", which includes chapters on Reviews, Definition, History, Classification, Pathologic Anatomy, Chemical Composition, Incidence, Pathogenesis, Clinical Course, Complications, Diagnosis, Prognosis and Therapy. The Classification includes those of Goodall, Barzilai, Schiller, Novak, Ten Teacher, Miller, Geist and Taylor which are presented in detail. The remainder of the book considers Para-ovarian Tumors, Endocrine Tumors, Chorio-epitheliomas, Common Cysts, Common Carcinomas (including Krukenberg), Mesonephromas and Brenneromas, Endometriosis, Teratoids, and Non-epithelial Tumors in the same systematic way, with some additional material included. There are abundant good illustrations to supplement the reading material. The illustrations are generally excellently reproduced. A worthy contribution includes a discussion under each heading of similar ovarian tumors in animals.

Careful reading of the text is required to obtain an adequate idea of the immensity of the undertaking. It is truly an encyclopedia of facts. The accumulated knowledge brought together by Selye is far greater than ever before attempted in the English language, so far as the reviewer has observed, and written in such a manner that one immediately senses the patience, ingenuity, literary and scientific ability of the author. The Encyclopedia, as it refers to Ovarian Tumors, will serve as a valued addition to the reference library of all those interested in the gynecology, endocrinology and pathology of the subject.

Detroit

D. C. BEAVER



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